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L.R. Leon

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Thermal and Mountain Medicine Division
U.S. Army Research Institute of Environmental Medicine
Natick, MA 01760-5007

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Heat stroke is a life-threatening illness that affects all segments of society. The etiology of the long-term consequences of this syndrome remains poorly understood such that preventive/treatment strategies are needed to mitigate its debilitating effects. Cytokines are important modulators of the acute phase response to stress, infection and inflammation. Despite several studies implicating cytokines in heat stroke pathophysiology, few studies have examined the protective effect(s) of cytokine antagonism on the morbidity and mortality of heat stroke. Heat shock proteins (HSPs) are highly conserved proteins that function as molecular chaperones for denatured proteins and reciprocally modulate cytokine production in response to stressful stimuli. A complex pathway of interactions between cytokines, HSPs and endotoxin is thought to be occurring in vivo in the orchestration of the APR to heat injury. This chapter provides an overview of current knowledge regarding cytokine, HSP and endotoxin interactions in heat stroke. Insight is provided into the potential therapeutic benefit of cytokine neutralization for mitigation of heat stroke morbidity and mortality based on our current understanding of their role in this syndrome.

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CHAPTER 24

Heat stroke and cytokines

Lisa R. Leon*

US Army Research Institute of Environmental Medicine, Thermal and Mountain Medicine Division, Natick, MA 01760-5007, USA

Abstract: Heat stroke is a life-threatening illness that affects all segments of society, including the young, aged, sick, and healthy. The recent high death toll in France (Dorozynski, 2003) and the death of high-profile athletes has increased public awareness of the adverse effects of heat injury. However, the etiology of the long-term consequences of this syndrome remains poorly understood such that preventive/treatment strategies are needed to mitigate its debilitating effects. Cytokines are important modulators of the acute phase response (APR) to stress, infection, and inflammation. Current data implicating cytokines in heat stroke responses are mainly from correlation studies showing elevated plasma levels in heat stroke patients and experimental animal models. Correlation data fall far short of revealing the mechanisms of cytokine actions such that additional research to determine the role of these endogenous substances in the heat stroke syndrome is required. Furthermore, cytokine determinations have occurred mainly at end-stage heat stroke, such that the role of these substances in progression and long-term recovery is poorly understood. Despite several studies implicating cytokines in heat stroke pathophysiology, few studies have examined the protective effect(s) of cytokine antagonism on the morbidity and mortality of heat stroke. This is particularly surprising since heat stroke responses resemble those observed in the endotoxemic syndrome, for which a role for endogenous cytokines has been strongly implicated. The implication of cytokines as mediators of endotoxemia and the presence of circulating endotoxin in heat stroke patients suggests that much knowledge can be gained from applying our current understanding of endotoxemic pathophysiology to the study of heat stroke. Heat shock proteins (HSPs) are highly conserved proteins that function as molecular chaperones for denatured proteins and reciprocally modulate cytokine production in response to stressful stimuli. HSPs have been shown repeatedly to confer protection in heat stroke and injury models. Interactions between HSPs and cytokines have received considerable attention in the literature within the last decade such that a complex pathway of interactions between cytokines, HSPs, and endotoxin is thought to be occurring *in vivo* in the orchestration of the APR to heat injury. These data suggest that much of the pathophysiologic changes observed with heat stroke are not a consequence of heat exposure, *per se*, but are representative of interactions among these three (and presumably additional) components of the innate immune response. This chapter will provide an overview of current knowledge regarding cytokine, HSP, and endotoxin interactions in heat stroke pathophysiology. Insight is provided into the potential therapeutic benefit of cytokine neutralization for mitigation of heat stroke morbidity and mortality based on our current understanding of their role in this syndrome.

*Corresponding author. Tel.: +1 508 233 4862;
Fax: +1 508 233 5298; E-mail: lisa.r.leon@us.army.mil

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Table 1. The heat illness continuum

Term	Definition
Heat cramps	Intermittent cramping pain in muscles subjected to strenuous activity; normal T_c; may occur in cold environment
Heat exhaustion (heat prostration; heat collapse)	Heat illness due to salt or water depletion resulting from strenuous physical exercise or prolonged exposure to a hot environment; T_c may or may not be elevated; decreased cardiac output
Heat stroke	Life-threatening illness characterized by $T_c 41°C$ and CNS abnormalities (delirium, fainting, seizures, and coma) resulting from prolonged exposure to a hot environment (classic) or strenuous physical exercise (exertional)

The heat illness continuum

The heat illness syndrome is typically depicted as a series of discrete events, characterized by pathophysiologic responses that increase in severity as one moves from the mildly innocuous condition of heat cramps to heat exhaustion and heat stroke (Table 1; Petersdorf, 1994). Heat cramps are the most benign condition, precipitated by strenuous muscle activity and profuse sweating that results in a loss of electrolytes. Spasms of skeletal muscles in the extremities may be sporadic, but painful in this condition (Wexler, 2002). Heat cramps typically occur following exercise in the cold and are not associated with elevated environmental temperature (Petersdorf, 1994). Heat exhaustion (also referred to as heat prostration or heat collapse) is the most common heat syndrome, which results from water or salt depletion in a hot environment. This mild to moderate illness is associated with an inability to maintain adequate cardiac output resulting in elevation of core temperature and potential for collapse. The use of diuretics and other medications may predispose individuals to heat exhaustion (Petersdorf, 1994). Heat stroke is the most serious condition resulting from prolonged exposure to a hot environment. The clinical definition of heat stroke includes core temperature in excess of 41.0°C, hot, dry flushed skin, and central nervous system (CNS) dysfunction (Petersdorf, 1994). While removal from the heat and rapid cooling are essential for heat stroke survival, a variety of complications ensuing after heat exposure make the choice of treatment modalities difficult, thus enhancing the probability of permanent neurological damage in survivors (Malamud et al., 1946; Dematte et al., 1998).

The absence or presence of an exertional component during heat exposure allows further categorization of heat stroke into its classic (i.e., passive) or exertional form. Classic heat stroke results from passive exposure to a hot environment and is typically observed in immunocompromised and aging populations, which show enhanced mortality during heat waves (Dematte et al., 1998; Naughton et al., 2002; Dorozynski, 2003). Pre-existing conditions, such as mental illness, alcoholism, or drug use (e.g., diuretics, anticholinergics) can predispose individuals to classic heat stroke (Levine, 1969; Naughton et al., 2002). Conversely, exertional heat stroke typically occurs in healthy, young individuals undergoing strenuous physical activity in hot environments. Athletes and soldiers represent two high-risk populations for this form of heat injury, although heat acclimatization of these populations can reduce risk (Coris et al., 2004). Exertional heat stroke is a particularly complicated heat syndrome to study since it is difficult to dissociate the direct effects of strenuous physical activity from that imposed by exposure to a hot environment.

Epidemiology of heat wave mortalities

In 1999, Chicago experienced its second deadliest heat wave in a decade in which 80 mortalities were reported (Naughton et al., 2002). These deaths occurred despite extensive programs to educate and provide interventions to high-risk populations,

such as the elderly. A 20-fold higher rate of heat-related mortality was reported in persons >75 years of age compared to younger populations (Naughton et al., 2002). Naughton et al. (2002) reported several social factors predisposing individuals to heat mortality, including living alone, an inability or unwillingness to leave one's home, residing on the top floor of buildings (heat rises) and an annual income of <$10,000/year (Naughton et al., 2002). A working air conditioner was shown to be the strongest protective factor, while fan cooling did not afford protection (Naughton et al., 2002). The availability and *use* of air conditioning units may be directly related to socioeconomic status, since these units are energetically expensive to run and in many cases are nonfunctional (Dematte et al., 1998; Naughton et al., 2002). Ineffective cooling prolongs exposure time to elevated ambient temperatures that increases the risk of mortality or permanent neurological damage in heat stroke survivors (Hart et al., 1982). The unwillingness of the elderly to leave their residences and the presence of higher indoor temperatures in the absence of air conditioning units (or refusal to use those units) magnifies the intensity of heat exposure. Vandentorren et al. (2004) suggest that the high death toll (~15,000) in the 2003 France heat wave may be partly explained by culture, as air conditioning units are typically not used in residences, retirement homes, or hospitals in that country. It is assumed that the large population (~10,000) of people aged 100 and over in France, in combination with a lack of air conditioning explains the high mortality rate in that country compared to other European regions that experienced the same heat wave (Dorozynski, 2003). As the average human lifespan increases, the development of education and intervention strategies will become increasingly important to prevent an increase in heat stroke deaths in the aged.

Urban structure also impacts heat mortality rates. Inhabitants of urban dwellings are exposed to greater intensity and longer duration of heat exposure since concrete structures do not effectively dissipate heat as nighttime temperatures decrease (Clarke, 1972). Landsberg (1970) reports city temperatures ~0.5° to 1.0°C warmer than rural areas; these "urban heat islands" represent the warmest areas which are typically in the center of the city and whose magnitude is dependent on city size. This relates to recent findings from Stott et al. (2004) demonstrating that activities that increase the production of greenhouse gases will double the risk for extreme climate fluctuations. Thus, as man's activities increase in urban centers it is not surprising that more heat deaths are reported, and expected in these areas (Shattuck and Hilferty, 1932, 1933).

Most heat waves occur across 3 or more days with the majority of hospitalizations and deaths occurring within 24 h of the onset of the event (Ramlow and Kuller, 1990; Kark et al., 1996; Dematte et al., 1998; Naughton et al., 2002). Thus, the duration of heat exposure and a lack of heat acclimatization increase the risk of heat stroke during the initial days of a heat wave. Several additional pre-disposing factors have been identified as increasing an individual's susceptibility to heat stroke mortality. Austin and Berry (1956) examined 100 cases of heat stroke and found cardiovascular illness in 84% of patients. Similarly, Levine (1969) found heat stroke mortalities in the elderly (200 cases) to be associated with arteriosclerotic heart disease (72%) and hypertension (12%). Animal studies have also shown a reduction in thermotolerance in spontaneously hypertensive rats (Wright et al., 1977). Heat strain imposes large cardiovascular demands on the body as blood flow is shunted from core organs to the skin to dissipate excess heat to the environment. A cardiac deficiency (e.g., congestive heart failure) impedes heat dissipation and an inability to maintain cardiac output during prolonged heat exposure leads to circulatory collapse and death. The high death rate (>600) in the Chicago 1995 heat wave was associated with hypertension, alcohol abuse, diuretic medications, aspirin, and pre-existing infections in the elderly population (Dematte et al., 1998). Alcohol depresses vasomotor reflexes and stimulates metabolism, resulting in increased heat gain and heat production. Thus, the high death toll due to excessive heat *per se* may be small compared to those caused by the aggravation in severity of a pre-existing condition. In addition, concurrent infections may predispose to heat stroke as individuals are immunocompromised prior to heat exposure (Knochel, 1989; Sonna et al., 2004).

Heat stroke causes changes in immune status such as disturbances in leukocyte distribution, production of cytokines, and bacterial translocation following gut ischemia that may predispose to early infection (Bouchama et al., 1992, 1993; Hall et al., 2001). The interaction of all of these events in heat stroke mortality only serves to complicate the etiology of this syndrome, thus limiting the success of currently established medical interventions.

Although soldiers and athletes represent young, healthy populations that do not have pre-existing physical ailments due to aging, significant hospitalization and death rates from exertional heat stroke have been recorded. Perhaps the most notorious example is the 1967 Six-Day War between Israel and Egypt in which 20,000 Egyptian soldiers suffered heat stroke deaths (Hubbard et al., 1982). Between 1911 and 1926, the US Navy reported 2049 cases of heat stroke deaths (Wakefield and Hall, 1927). Currently, the US Army hospitalizes 25–70 soldiers per 100,000 due to heat injury per year and the incidence of heat stroke hospitalizations has increased almost 10-fold over the past 20 years (Carter et al., 2005). During peacetime exercises, Malamud et al. (1946) noted that ~25% of fatal heat stroke cases occurred in military recruits that had been in training camp for less than ~2 weeks. The majority of cases occurred during the hottest summer months (typically July) and was likely a consequence of intense exercise with a lack of heat acclimatization. Obesity was also a significant factor (Malamud et al., 1946). Given the increasing incidence of obesity in the U.S., this may be a particular concern for future military populations. Carter et al. (2005) examined heat illness hospitalization rates and deaths for the US Army from 1980 to 2002 and reported the highest incidence in those recruits enlisted for <12 months, with recruits from northern, cold climate states at higher risk than those from southern, warm climate states.

Protective clothing may be a significant pre-disposing factor to heat stroke deaths in athletic and military populations. Under normal clothing conditions, sufficient heat exchange between the skin surface and the environment can occur to regulate core temperature within a narrow range, thus supporting thermal homeostasis. Protective clothing, which may consist of multiple layers and often encapsulates the head (a site of significant heat exchange; Rasch et al., 1991), forms an insulative layer of air between the skin and the environment, thus impeding heat exchange. Fifty-one cases of exertional heat illness were observed in military trainees in San Antonio, Texas, during participation in a 5.8 mile march in full battle dress uniform and boots (Smalley et al., 2003). Athletic uniforms also limit evaporative and convective heat loss during strenuous activity. Similar to that observed with heat waves and military training exercises, a lack of acclimatization to the uniform and high environmental temperatures results in the majority of heat stroke cases observed on the second or third day of football practice in hot weather (Graber et al., 1971; Roberts, 2004). To avoid heat exposure, exercise regimens are typically scheduled for the early morning hours, when it is cooler; guidelines for adequate hydration are also implemented in athletic and military populations (Armstrong et al., 1996; Departments of Army and Air Force, 2003). Paradoxically, the use of nutritional supplements and implementation of fluid replacement guidelines may result in longer duration of exposure to elevated temperatures, potentially increasing heat stroke incidence (Carter et al., 2005). A lack of heat acclimatization as well as increased emphasis on high intensity, long-duration exercise regimens, which adds a metabolic heat load to the body, has likely increased heat stroke hospitalization rates in military and athletic populations in the past two decades (Carter et al., 2005). To further complicate the issue, exercise has been shown to induce similar changes in core temperature (values as high as 41.9°C have been noted in marathon runners; Maron et al., 1977) and immune parameters (Cross et al., 1996; Nielsen and Pedersen, 1997) as heat exposure such that teasing apart the influence of these two factors in exertional heat stroke is made more difficult.

Systemic responses to heat stroke

Heat stroke is clinically defined as core temperature in excess of 41.0°C, hot, flushed dry skin and CNS dysfunction, such as delirium, coma, and

seizures (Petersdorf, 1994). However, a myriad of thermoregulatory, hemodynamic, and organ abnormalities are manifest in heat illness beyond those immediately recognized by the clinical definition of this syndrome (Table 2). The implication of cytokines as regulators/modulators of several of the responses listed in Table 2 suggests that neutralization of one or more of the actions of these endogenous mediators may be beneficial in the mitigation of heat stroke morbidity and mortality.

Despite the myriad of complications associated with heat illness, an elevation of core temperature above 41.0°C (often referred to as fever or hyperpyrexia) is the most widely recognized symptom of this syndrome. Core temperature is extremely labile to environmental and physiological perturbations and is simple to measure, thus providing rapid and powerful information regarding homeostatic balance of the individual. The basis for a specific core temperature cut-off value of 41°C as a heat stroke criterion is not readily apparent, but may reflect an attempt to dissociate the degree of hyperthermia observed in heat illness from that of infection, in which fevers rarely exceed 41°C (Dubois, 1949).

Core temperature varies dramatically in heat stroke patients with ranges of 41° to 42°C commonly observed and values as high as ~47°C reported (Bouchama et al., 1991, 1993; Chang, 1993; Hammami et al., 1997; Hashim et al., 1997; Lu et al., 2004; Sonna et al., 2004). Large variability in core temperature values may be due to several factors, including (1) differences in the time of clinical presentation such that patients' temperature is obtained at varying stages of heat stroke progression and treatment (i.e., hyperthermia vs. cooling), (2) individual differences in the critical thermal maximum (CTM) associated with heat stroke mortality, and (3) the site of the temperature measurement. CTM is defined as the minimum core temperature that is lethal to an organism (Cowles and Bogert, 1944; Hutchison, 1961). As previously discussed predisposing factors such as medications, infection, and cardiovascular disease may enhance susceptibility to heat stroke, which would presumably manifest as a lower CTM prior to collapse. Attempts to define the CTM in several species indicate wide species and

Table 2. Complications associated with heat stroke

Thermoregulatory
T_c41°C (hyperpyrexia)
Hot, dry skin
Fever (long-term symptom)
Hypothermia (animal studies only)

Hemodynamic
Dehydration/hemoconcentration
Disseminated intravascular coagulation (DIC)
Elevated C-reactive protein (CRP)
Endotoxemia
Hyperglycemia/hypoglycemia
Hypotension/cardiovascular abnormalities
Increased cortisol/corticosterone
Increased pro- and anti-inflammatory cytokines
Lactic acidosis
Leukocytosis
Rhabdomyolysis

Tissue injury
Adrenal ischemia
Cardiac focal necrosis
Cerebral edema/ischemia
Increased intestinal permeability
Liver necrosis (long-term symptom)
Lung edema
Renal failure
Spleen necrosis
Multi-organ system failure (ultimate cause of mortality)

inter-individual variability. Austin and Berry (1956) report core temperature values ranging from 38.5 to 44.0°C in heat stroke patients with 10% of the reported mortalities occurring below 41.1°C. Thus, many patients do not meet the clinical core temperature criterion of heat stroke.

Obviously, it is not ethical or desirable to experimentally determine the CTM of humans. Therefore, animal models of heat stroke have been developed to more precisely determine the CTM in various species in order to study the mechanisms of thermoregulatory control during heat exposure. Adolph (1947) determined the CTM in cats (~43.5°C), dogs (41.7°C), and rats (42.5°C), proposing differences in tissue susceptibility as responsible for variability in CTM between species (tissue injury was not measured in this study). Wide ranges of CTM are reported in monkeys (~44.5°C; Gathiram et al., 1987a), dogs (37.7° to 41.1°C; Drobatz and Macintire, 1996),

sheep (43.7° to 44.0°C; Hales et al., 1987), rats (40.4° to 45.4°C; Ohara et al., 1975; Hubbard et al., 1976; Wright et al., 1977; DuBose et al., 1983a; Lord et al., 1984), mice (42.7°C and ~44° to 45°C; Wright, 1976; Leon et al., 2005), and salamanders (~33°C; Hutchison and Murphy, 1985). Malamud et al. (1946) proposed direct thermal injury to the thermoregulatory centers of the brain as the primary mechanism of heat stroke mortality, despite an inability to detect thermal injury to the hypothalamus at autopsy of 125 fatal cases of heat stroke. The ability to induce core temperatures of 41.6° to 42.0°C in humans (within the reported CTM range) with no adverse clinical effects illustrates the inability to rely on a specific CTM for injury predictions (Bynum et al., 1978). Similarly, rectal temperatures of 41.9°C have been recorded in competitive runners showing no adverse clinical signs of heat injury, suggesting that this level of elevated core temperature is tolerable in humans (Maron et al., 1977). Of course, runners may be acclimatized to elevated core temperatures due to repeated exposure during intensive training regimens.

Inconsistency in CTM values may be due as much to individual and species variability in thermal resistance as to methodological differences between studies. One of the most dramatic differences between studies is the ambient temperature used to induce heat stroke. The ambient temperature used in animal studies ranges from 38.6 to 59.4°C, making comparisons between studies difficult (Adolph, 1947; Ohara et al., 1975; Wright, 1976; Hubbard et al., 1977; Wright et al., 1977; DuBose et al., 1983a; Gathiram et al., 1987a; Leon et al., 2005). The physiological relevance of 59.4°C is questionable since this ambient temperature is not routinely encountered in nature (Adolph, 1947). Similarly, the majority of these studies exposed animals to pre-heated environmental chambers (Adolph, 1947; Ohara et al., 1975; Wright, 1976; Hubbard et al., 1977; Wright et al., 1977; DuBose et al., 1983a; Gathiram et al., 1987a; Heidemann et al., 2000), which represents a heat "shock" rather than heat "stress" paradigm. *In vitro* studies have also used heat shock paradigms (e.g., 42° to 43°C water bath exposure for 1 h) to examine responses in different cell types (D'Souza et al., 1994; Watanabe et al.,

1997, 1998). Heat stroke severity is influenced by the rate of heating such that rapid exposure to a pre-heated chamber may not provide sufficient lag time for the thermoregulatory system to sense and respond to a dramatic shift in ambient temperature before core temperature reaches lethal levels (Hutchison, 1961; Flanagan et al., 1995). Thus, the physiological relevance of the heat shock paradigm is questionable.

Core temperature values of heat stroke patients will differ depending on the site of measurement. In humans, esophageal temperature is the most accurate and responsive to changes in blood temperature, although instrumentation may not be feasible in severely injured, unresponsive patients. Rectal temperature has a slower response rate and gives slightly higher readings than esophageal temperature (Bynum et al., 1978). Oral temperature is rapidly measured, but may provide inaccurate (low) readings due to hyperventilation in the heat stroke patient (Cole, 1983). The recent development of remote core temperature sensing by radiotelemetry is a powerful technique that is applicable to both human and animal studies. In humans, core temperature may vary as the pill is swallowed and travels through the gastrointestinal (GI) tract. In animal models, the use of radiotelemetry has significantly improved experimental design by permitting an assessment of rapid and long-term core temperature changes induced by heat exposure of conscious, freely moving animals (Leon et al., 2005). It is anticipated that these advances in physiological monitoring will significantly improve our ability to model heat stroke responses in experimental test species.

Thermoregulatory consequences of heat stroke

While several characteristics of the thermoregulatory response during progression to heat stroke collapse are well defined, the core temperature response observed during recovery has received less attention. This is rather surprising since the magnitude, duration, and direction of core temperature changes displayed during recovery may provide information regarding severity and etiology of the initial heat insult. In experimental animals, hypothermia is the

Table 3. Incidence of hypothermia during heat stroke recovery

Species	Maximum T_c	Recovery T_a	Hypothermia	Authors
Cat	NS	NS	33.5, 35.0°C	Adolph (1947)
Guinea pig	NS	NS	37°C	Adolph (1947)
	IPH; 43.9°C	21° to 23°C	T_h; 34°C	Romanovsky and Blatteis (1996)
Mouse	42°C	10, 25°C	32°C	Wright (1976)
	38.7° to 41.9°C	24°C	29° to 35°C	Wilkinson et al. (1988)
	42.4, 42.7, 43.0°C	25°C	29° to 31°C	Leon et al. (2005)
Rat	41.5° to 42.8°C	Heat pad 39° to 40°C	35° to 36°C	Lord et al. (1984)
Salamander	33.7°C	Thermal gradient	10°C below controls	Hutchison and Murphy (1985)

Abbreviations: IPH — intraperitoneal heating; T_a — ambient temperature; T_c — core temperature; T_h — hypothalamic temperature; NS — not specified.

predominant heat stress recovery response (Table 3). Heat-induced hypothermia is the term used to define the seemingly paradoxical decrease of core temperature below baseline levels (Romanovsky and Blatteis, 1996). Note that the depth of hypothermia varies widely between studies, which may be species-specific or a result of nonconformity between experimental designs. Nevertheless, regardless of the experimental conditions, hypothermia of $>1.0°C$ is commonly observed in both mammals and poikilotherms (e.g., salamanders; animals without effective autonomic temperature regulation; IUPS, 2001). In mice, hypothermia is quite profound such that core temperature may be regulated only a few degrees above ambient temperature (Wright, 1976; Wilkinson et al., 1988; Leon et al., 2005). The depth of hypothermia is also affected by the ambient temperature to which animals are exposed during recovery. As shown in Fig. 1, the hypothermic response of mice during heat stress recovery is significantly blunted during exposure to a thermal gradient, which allows the behavioral selection of a wide range of ambient temperatures, compared to that observed during housing at a constant temperature of 25°C (cool ambient temperature for mice). The implication of these findings to heat stroke recovery responses in humans is currently unknown, although rapid cooling (which would be facilitated at lower ambient temperatures) is the most effective therapy for recovery.

In mice, the depth ($\sim$1.0° to 5.0°C) and duration ($\sim$1–24 h) of hypothermia is directly related to the severity of the heat insult, suggesting that core temperature responses displayed during recovery may serve as sensitive biomarkers of injury (Wilkinson et al., 1988; Leon et al., 2005). This is also apparent in comparisons of individual responses to heat stress; those animals experiencing the longest duration of heat exposure typically show the most profound heat-induced hypothermic response during recovery (Fig. 2). Exposure to a recovery ambient temperature that prevents hypothermia development enhances heat-induced intestinal damage and significantly decreases survival in mice (Wilkinson et al., 1988; Leon et al., 2005).

The connection between hypothermia and tissue injury suggests that cooling of heat stroke patients to a hypothermic level (i.e., core temperature $<37°C$) may be beneficial for the prevention of tissue injury. Further support for this contention is provided by the use of induced hypothermia, in which core temperature is physically decreased using cooling blankets or other methods, as a protective measure during cardiopulmonary bypass surgery and as treatment for cerebral ischemia and stroke (Marion et al., 1997; Dietrich and Kuluz, 2003). The realization that hypothermic treatment would be more efficacious if regulated, rather than forced reductions in core temperature were implemented suggests that further studies are required to determine the regulated nature of hypothermia under injurious conditions (Gordon, 2001). If cytokines are regulators/modulators of heat-induced hypothermia or their production is influenced by hypothermia, as previously described for
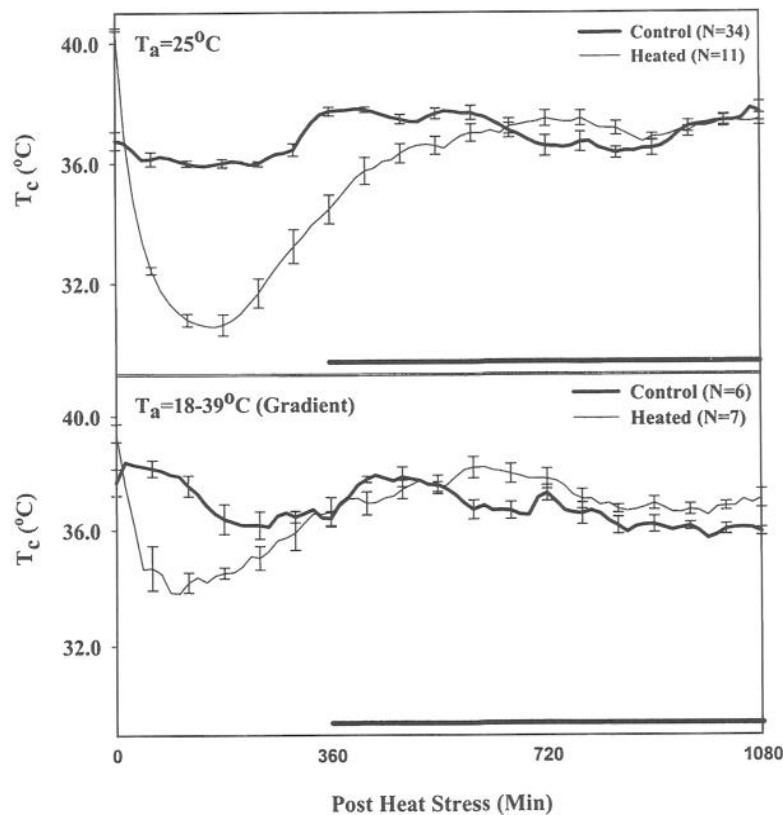
Fig. 1. Twenty-four hour core temperature ($\pm 0.1^\circ$C) response of male C57BL/6J mice during heat stress recovery at a constant ambient temperature (T_a) of $25\pm2^\circ$C (top) or in a thermal gradient that allows the behavioral selection of a wide range of ambient temperatures (18° to 39°C; bottom). Mice were heat stressed at an ambient temperature of $39.5\pm0.5^\circ$C, in the absence of food and water, until their core temperature reached 42.4°C (data not shown; Leon et al., 2005). Control mice were not exposed to heat stress. Immediately following heat exposure, mice were placed at 25°C or in the thermal gradient for recovery observations. Note that the hypothermic response displayed within the first $\sim$360 min of recovery is significantly more pronounced at 25°C than in the thermal gradient. Black horizontal bar represents lights-off period in a 12:12 h L:D cycle. Sample sizes are indicated in parentheses. Core temperature was collected at 1 min intervals in conscious, freely moving mice using intraperitoneally implanted radiotelemetry devices (Data Sciences International, St. Paul, MN).

bacterial infection, they may represent one class of substances that could be targeted to induce hypothermia in a regulated fashion and minimize tissue injury in heat stroke patients (Arons et al., 1999; Fairchild et al., 2004; Leon et al., 2006).

In humans, anecdotal evidence suggests that fever is a symptom of heat stroke, persisting for 7–14 days following clinical presentation in some patients (Malamud et al., 1946; Austin and Berry, 1956; Attia et al., 1983). The occurrence of this thermoregulatory response late in the heat stroke syndrome suggests that fever, which is a tightly controlled physiologic response to stress, is more

directly related to the complications ensuing after heat exposure, than to the initial heat insult. Although "fever" is reported immediately upon admission in many reports, it is likely that this represents the hyperthermic response to direct heat exposure rather than a true fever. Fever is defined as a regulated increase in the hypothalamic thermal setpoint and is observed in response to several stimuli including bacterial infection, stress, and tissue inflammation (Kluger, 1991). Fever results from the coordinated action of behavioral and physiological mechanisms that increase heat production and decrease heat loss to raise core
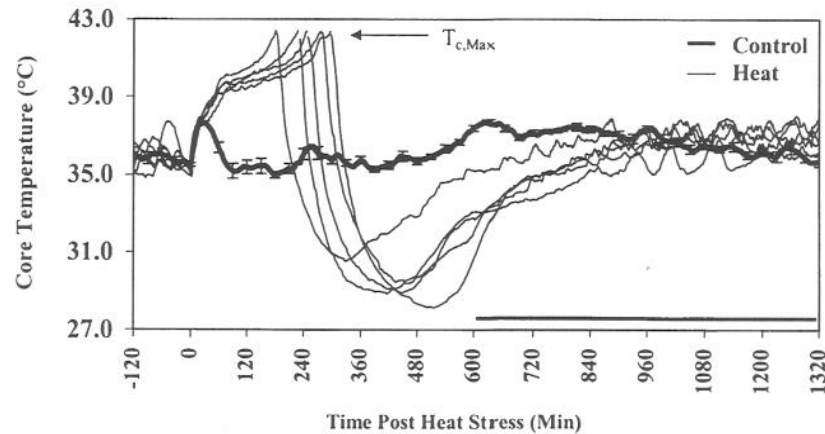
Fig. 2. Core temperature response (± 0.1°C) of male C57BL/6J mice during heat exposure (from time 0 to $T_{c,Max}$) and recovery at a constant ambient temperature of 25 ± 2°C (from $T_{c,Max}$ to 1320 min). $T_{c,Max} = 42.4$°C. All mice served as their own controls. During the control condition, mice remained in their original cage location in the animal room and were not exposed to heat stress. Details of the heat stress protocol have been described elsewhere (Leon et al., 2005). Note that those mice experiencing the longest duration of heat exposure displayed the lowest depth and duration of hypothermia. Black horizontal bar represents lights-off period in a 12:12 h L:D cycle. Core temperature was collected at 1 min intervals in conscious, freely moving mice using intraperitoneally implanted radiotelemetry devices (Data Sciences International, St. Paul, MN).

temperature to a new elevated level. The presence of tissue injury and endotoxemia (both described in more detail below) in heat stroke suggests that the persistence of fever beyond the initial day of heat exposure (and clinical admission) may be a result of a systemic inflammatory response. This may also account for fever not being widely recognized as a heat stroke recovery response in animal studies. Due to reliance on rectal probes, restraint, and/or anesthesia for core temperature measurements, thermoregulatory responses to heat stress have typically not been examined across multiple circadian cycles in animal models (Lin et al., 1994, 1997; Romanovsky and Blatteis, 1996; Bouchama et al., 2005). The advent of radiotelemetry has alleviated this experimental limitation and shown that a "fever-like" core temperature elevation is observed 24–36 h following heat exposure in mice (Leon et al., 2005). Interestingly, mice show virtually identical increases in the fever-like core temperature response (~ 1.0° to 1.5°C) irrespective of heat severity (Leon et al., 2005). This is in direct contrast to hypothermia, which is directly related to heat severity in mice, as previously described (Wilkinson, et al., 1988; Leon et al., 2005), indicating that fever is not a reliable biomarker of heat severity. Interestingly, mice that do

not recover from hypothermia and develop fever, succumb to heat stroke (Leon et al., 2005). It is unclear if this is indicative of a protective function of fever or a debilitating effect of prolonged hypothermia in this syndrome.

Hemodynamic changes

Significant hemodynamic alterations occur in heat stroke patients (Table 2). Evaporative cooling (sweating in humans, salivary spreading in rodents) is a primary mechanism of core cooling under heat stress conditions. Prolonged heat exposure can induce significant dehydration and hemoconcentration. Disturbances in plasma glucose homeostasis (i.e., hyper- or hypoglycemia) are also common in heat stroke and may be directly related to thermal injury of the liver (Bouchama et al., 1996). Phosphoenolpyruvate carboxykinase (PEPCK) is a key regulatory enzyme of the hepatic gluconeogenic pathway — alterations in PEPCK regulation following thermal injury to the liver have been hypothesized as a mechanism of heat-induced hypoglycemia, although this has not been experimentally confirmed (Paidas et al., 2002). Previous reports showing an ability of dehydration
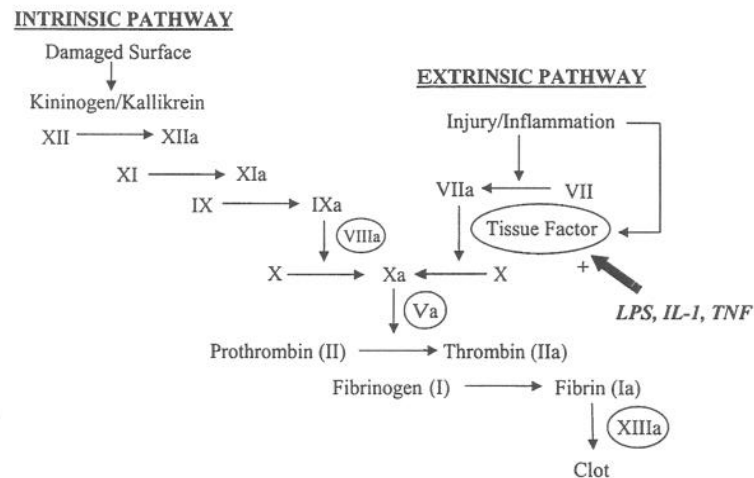
and hypoglycemia to induce hypothermia in small rodents suggest that these pathophysiologic changes may represent two, of perhaps several, physiological stimuli driving the development of heat-induced hypothermia in rodents (Buchanan et al., 1991; Ibuka and Fukumura, 1997); the implications of this for the human condition is unknown. Immune dysfunction is common with disturbances in the distribution of several peripheral lymphocyte subpopulations (Bouchama et al., 1992; DuBose et al., 2003). The degree of hyperthermia is directly correlated with increased lymphocytes and T suppressor-cytotoxic cells (Bouchama et al., 1992). Changes in regional blood flow, increased catecholamine and cortisol release, and direct effects of exercise, cytokines, and endotoxin are proposed mechanisms of increases in these cell types with heat stroke (Levine, 1969; Maron et al., 1977; Haynes and Fauci, 1978; Al-Hadramy, 1989; Bouchama et al., 1991, 1992; Kappel et al., 1991; Frisina et al., 1994; Cross et al., 1996; Nielsen and Pedersen, 1997; Mitchell et al., 2002; Dubose et al., 2003).

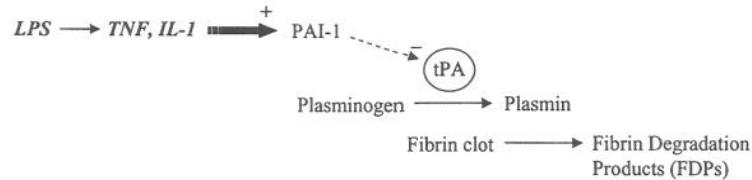### Disseminated intravascular coagulation (DIC)

Disseminated intravascular coagulation (DIC) is a systemic intravascular disorder resulting from uncontrolled activation of coagulation and/or impairment of fibrinolysis or anticoagulation (Fig. 3; Bouchama et al., 1996; Bouchama and Knochel, 2002). Studies of the sepsis syndrome have provided the majority of data regarding DIC pathway components that are affected by cytokines. Cytokine modulation of DIC is supported by several lines of evidence including: (1) increased plasma levels of interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF) in patients with DIC — with high IL-6 correlating with organ failure (Wada et al., 1991a, b, 1993), (2) alteration of coagulation following IL-1, IL-2, IL-6, IL-8, IL-10, IL-12, or TNF injection (van der Poll et al., 1990; Baars et al., 1992; Pradier et al., 1993; Jansen et al., 1995; Stouthard et al., 1996; Neumann et al., 1997), and (3) attenuation of coagulation following injection of cytokine neutralizing antibodies (Paleolog et al., 1994; van der Poll et al., 1994; Schmid et al., 1995).

There are several different components of the coagulation, fibrinolytic, and anticoagulation pathways that have been shown to be affected by cytokines and endotoxin in the sepsis syndrome (Fig. 3). Tissue factor (TF) is a cell surface receptor expressed by monocytes and vascular endothelial cells; increased expression of TF results in initiation of the coagulation cascade via the extrinsic pathway (Fig. 3). TF expression is induced following exposure to blood; thus major trauma, burns, hereditary vascular or chronic inflammatory disorders increase an individual's susceptibility to DIC via increased TF expression (Baker, 1989; Bakhshi and Arya, 2003). The expression of TF is regulated by endotoxin, as well as pro- and anti-inflammatory cytokines with TNF, IL-1α, IL-1β, IL-6, IL-8, leukemia inhibitory factor, interferon (IFN)γ, and monocyte chemoattractant protein (MCP)-1 stimulating TF and TGFβ, IL-4, IL-10, and IL-13 inhibiting its expression (Herbert et al., 1992; Pradier et al., 1993; Schwager and Jungi, 1994; Del Prete et al., 1995; Neumann et al., 1997). In addition to affecting the extrinsic pathway of coagulation, endotoxin and cytokines modulate the fibrinolytic and anticoagulation pathways. The fibrinolytic pathway is a natural anticoagulant pathway that is important for host protection against excessive clotting. Data from human and animal experiments suggest that cytokines mediate lipopolysaccharide (LPS)-induced increases in plasminogen activator inhibitor (PAI)-1 in several organs, including liver, kidney, and lung (Fig. 3B; Sawdey and Loskutoff, 1991). PAI-1 is a negative inhibitor of tissue plasminogen activator (tPA), an essential enzyme involved in fibrin clot degradation (Fig. 3B). High PAI-1 levels have been shown to precede DIC incidence and correlate with poor outcome; PAI-1 knockout mice are resistant to LPS-induced kidney thrombosis (Sawdey and Loskutoff, 1991; Yamamoto and Loskutoff, 1996). Finally, cytokines can also modulate the protein C–protein S anticoagulation pathway at several levels, preventing proteolytic cleavage of several factors (Va and VIIIa) involved in the coagulation pathway (Fig. 3C; Redl et al., 1995; Yamamoto et al., 1999). The reader is referred to reviews that discuss the intricacies of cytokine modulation of these pathways with DIC (Levi, 2001; van der Poll et al., 2001).

A. Coagulation Pathway

INTRINSIC PATHWAY

Damaged Surface

Kininogen/Kallikrein

XII ⟶ XIIa

XI ⟶ XIa

IX ⟶ IXa

(VIIIa)

EXTRINSIC PATHWAY

Injury/Inflammation

VIIa ⟵ VII

(Tissue Factor)

X ⟶ Xa ⟵ X

(Va)

+

LPS, IL-1, TNF

Prothrombin (II) ⟶ Thrombin (IIa)

Fibrinogen (I) ⟶ Fibrin (Ia)

(XIIIa)

Clot

B. Fibrinolytic Pathway

LPS ⟶ *TNF, IL-1* ⟹ + PAI-1 ⤍ (tPA)

Plasminogen ⟶ Plasmin

Fibrin clot ⟶ Fibrin Degradation Products (FDPs)

C. Anticoagulant Pathway

LPS, IL-1, TNF

(TM:T)

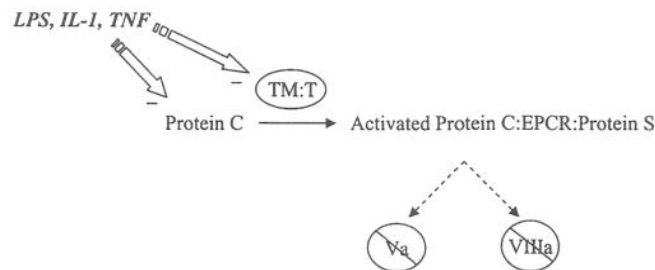Protein C ⟶ Activated Protein C:EPCR:Protein S

(Va) (VIIIa)

Fig. 3. Coagulative (A), fibrinolytic (B), and anticoagulative (C) pathways involved in disseminated intravascular coagulation. The coagulation cascade generates a fibrin clot through intrinsic and/or extrinsic pathways. LPS, IL-1, and TNF have been shown to elicit tissue factor formation. This leads to formation of factor Va–Xa and VIIIa–IXa complexes, which result in the generation of thrombin and clot formation. The fibrinolytic pathway is activated by LPS via the production of TNF and IL-1. Cytokine activation of plasminogen activator inhibitor (PAI)-1 results in inhibition of tissue plasminogen activator (tPA), which impairs fibrinolysis. LPS, IL-1, and TNF also affect the anticoagulation pathway through downregulation of thrombomodulin (TM) expression, which ultimately prevents the inactivation of factors Va and VIIIa, thus prolonging clot formation.

Tissue injury responses

Heat-induced tissue damage may be extensive, as injury to the liver, kidney, spleen, heart, lung, small intestine, brain, and skeletal muscle (rhabdomyolysis) is commonly observed in human and animal studies (Malamud et al., 1946; Graber et al., 1971; Bouchama et al., 1996, 2005; Dematte et al., 1998;

Lu et al., 2004). The severity of heat stroke is purported to be primarily related to the extent of damage incurred in the CNS, liver, and kidneys (Kew et al., 1967, 1970). The extent of peripheral organ damage is most readily assessed by analysis of serum enzyme levels, such as creatine phosphokinase (CPK; skeletal muscle), uric acid (kidney), alanine aminotransferase (ALT; liver), and aspartate amino transferase (AST; liver). However, due to plasma levels of these enzymes being altered by heat as well as exhaustive exercise (in the absence of heat stroke), a differential diagnosis of heat stroke is not always possible based on these measures alone.

Tissue histopathology studies have provided detailed analysis of the extent of thermal damage to several organs in heat stroke cases. GI barrier dysfunction is a common complication of heat exposure (Moseley et al., 1994; Lambert et al., 2002, 2004). Prolonged heat exposure induces a reduction in splanchnic blood flow as a greater proportion of cardiac output is shunted to the skin to facilitate heat dissipation. Resultant GI ischemia (with subsequent cytokine and free-radical production) contributes to barrier disruption, which is commonly observed as dilation of the central lacteals of intestinal villi (Figs. 4A, B; Hall et al., 2001; Lambert et al., 2002). Renal failure is a common complication of heat stroke, as characterized by glomerular ischemia and hemorrhages (Malamud et al., 1946; Chao et al., 1981; Bouchama et al., 1996). Tubular necrosis is also common in human and animal heat stroke models (Figs. 4C, D); protein clumping in tubular epithelial cells of the kidney is thought to be a consequence of direct thermal injury, rhabdomyolysis, or DIC (Kew et al., 1967; Graber et al., 1971; Raju et al., 1973; Chao et al., 1981; Kilbourne et al., 1982; Lu et al., 2004; Carter et al., 2005). Cytoplasmic protein clumping in the spleen is thought to be a direct result of hyperthermia as the organs are essentially "cooked and coagulated" (Figs. 4E, F; Chao et al., 1981). Fatty change is observed in the liver of exertional heat stroke cases and may be a consequence of enhanced breakdown of fats induced by hyperthermia and/or an inability of the liver to mobilize the fat (Chao et al., 1981). Typically, liver injury is seen in long-term survivors, suggesting that it may be a consequence of the

inflammatory response that ensues during recovery, rather than representing an acute (immediate) response to hyperthermia (Malamud et al., 1946). This has also been observed in a mouse model of passive heat exposure in which liver damage was absent through 24 h of recovery, but appeared ~72 h after the initial heat insult (Fig. 5). The presence of circulating endotoxin in some heat stroke patients is thought to be due to leakage following GI barrier disruption, but may also be related to liver damage since this organ is one of the major sites of endotoxin clearance (Bradfield, 1974; Nolan, 1981). Similarly, renal failure has been suggested as a potential mechanism for increased cytokine (i.e., soluble TNF receptor) concentrations, as cytokine clearance is a reported function of this organ (Hammami et al., 1997). Ultimately, multi-organ system dysfunction is the cause of heat stroke mortality and is revealed at autopsy as edema and micro-hemorrhages in several organs of the periphery as well as specific brain regions (Malamud et al., 1946; Chao et al., 1981).

Cytokines and heat stroke

> Although shock undoubtedly plays a significant role in the course of heat stroke…it is regarded as a secondary manifestation and therefore non-specific and unessential to the fundamental pathogenesis of the disorder.
> Malamud et al., 1946

The heat illness syndrome is a continuum of increasing severity that is inclusive not only of the conditions incurred during direct heat exposure, but also those pathophysiologic responses that manifest during long-term recovery. As such, multi-organ system failure is now thought to result from heat cytotoxicity in combination with a subsequent systemic inflammatory response syndrome (SIRS) of the host to tissue injury (Bouchama and Knochel, 2002). Based on this realization, Bouchama and Knochel (2002) proposed a new definition of heat stroke as "a form of hyperthermia associated with a systemic inflammatory response leading to a syndrome of multi-organ dysfunction in which encephalopathy
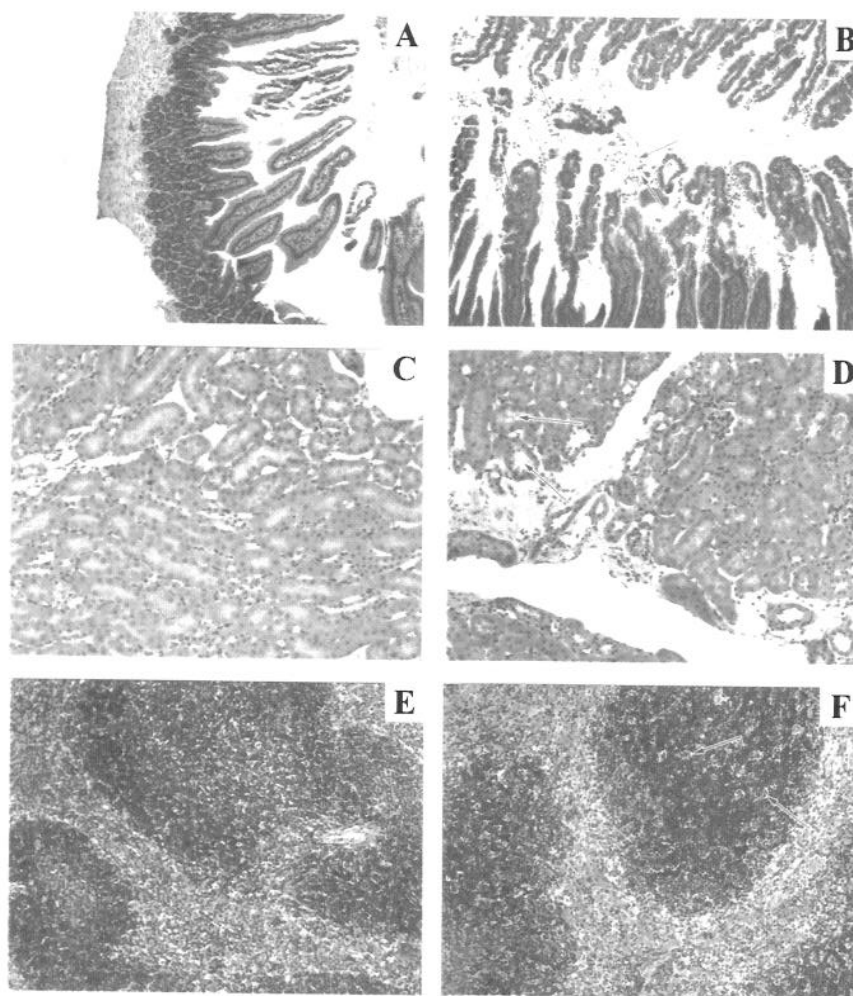
Fig. 4. Representative photomicrographs of small intestine (top), kidney (middle), and spleen (bottom) obtained from male C57BL/6J mice exposed to a control (A, C, E) or heat stress condition (B, D, F). Tissues are from male C57BL/6J mice heat stressed to a maximum core temperature of 42.7°C and allowed to recover until hypothermia, at which time tissues were collected (Leon et al., 2005). Tissues were stained with hematoxylin and eosin for microscopic evaluation at magnification 200 ×. Arrows indicate identified tissue lesions, which are described in the text.

predominates." Note the lack of inclusion of a specific core temperature value in this definition, which may be reflective of the wide variability of core temperature responses observed in heat stroke cases.

Endogenous cytokines may be important mediators of SIRS in heat stroke patients. Cytokines are intercellular chemical messengers released by a variety of cell types, including macrophages, T and B cells, endothelial cells, and astrocytes (Kelker et al., 1985; Chensue et al., 1989; Van Dam et al.,

1992; Sharif et al., 1993; Malyak et al., 1994). Their defining characteristics include a general lack of constitutive production and pleiotropy or redundancy of actions. This latter feature has important implications since it is rare that a cytokine is released in the absence of other endogenous substances that may influence its action. Furthermore, antagonism of the physiological actions of one cytokine may be compensated by overlapping properties of a different, perhaps related cytokine. Different classes of cytokines have redundant
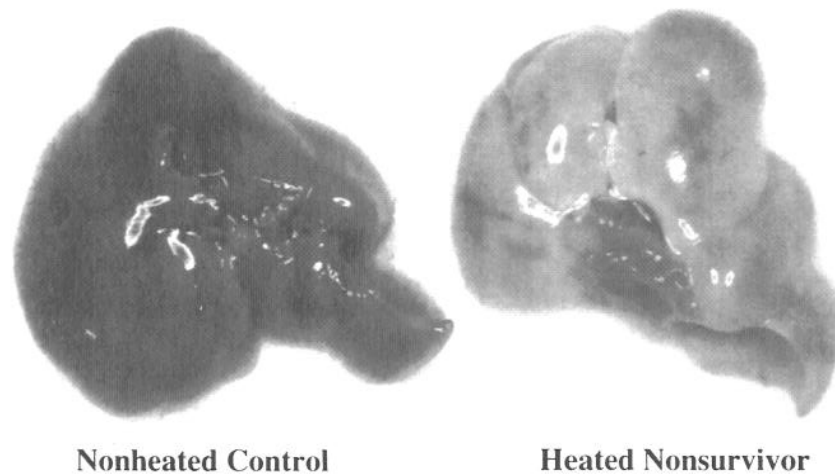
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Nonheated Control   **Heated Nonsurvivor**

Fig. 5. Photograph showing the "fatty liver" change observed in mice at ~72 h following heat stress. Livers from a nonheated control (left) and heat stressed nonsurvivor (right) are shown.

effects on specific cell types and combinations of cytokines can be synergistic or antagonistic, depending on the targeted cell types and the combination of cytokines that are present (the cytokine "milieu"). Several pro- and anti-inflammatory cytokines are elevated concomitantly in the circulation of heat stroke patients. However, due to an examination at end-stage heat stroke or at clinical presentation (often after cooling has occurred), our understanding of changes in the balance of pro- and anti-inflammatory cytokines over long-term progression of this syndrome remains poorly understood. Although attempts have been made to classify particular cytokines as more harmful than others in the morbidity and mortality of human heat stroke (e.g., IL-6), these efforts have been hindered by a lack of data beyond that provided by correlation studies.

Cytokine-inducing stimuli include bacterial and viral infection (Patel et al., 1994; Drexler, 1995), psychological stress (Maes et al., 2000; Oka et al., 2001), heat stress or whole body hyperthermia (WBH; Neville and Sauder, 1988; Bouchama et al., 1991, 1993, 2005; Lin et al., 1994; Haveman et al., 1996), exercise (Camus et al., 1997; Moldoveanu et al., 2001; Nieman et al., 2001; Suzuki et al., 2003), and other cytokines (Content et al., 1985; Neta et al., 1992). Note that heat stroke patients may be exposed to several of these stimuli

concomitantly, complicating etiology of their condition. Heat exposure influences and/or induces a variety of physiological responses that are known to be modulated by endogenous cytokines, including fever (Kluger, 1991; Leon et al., 2005), hypothermia (Romanovsky and Blatteis, 1996; Leon, 2004, 2005), increased gut permeability (Moseley et al., 1994; Hall et al., 2001; Oshima et al., 2001; Desai et al., 2002; Lambert et al., 2002; Wang and Hasselgren, 2002), activation of the hypothalamic-pituitary-adrenal (HPA) axis (e.g., glucocorticoid release; Berkenbosch et al., 1987), and hypotension (Lin et al., 1997).

In order to clearly delineate a role for an endogenous cytokine in heat stroke, a series of criteria need to be experimentally satisfied (adapted from Kluger, 1991). While these criteria were originally described for characterization of endogenous cytokines in fever, they can be similarly applied to the study of heat stroke pathophysiologic responses. Briefly, a cytokine should elicit the expected response(s) when injected or infused (*Criterion 1*), its endogenous release/production should be temporally correlated with the appearance of heat stroke symptoms (*Criterion 2*), and antagonism of a cytokine's action or production should inhibit or eliminate the physiological symptom (*Criterion 3*). While the first two criteria provide evidence in favor of a role of the targeted protein in a response,

efficacy provided by protein neutralization is the most stringent evidence in favor of that endogenous substance playing a role in the response of interest. Surprisingly few studies have examined the effectiveness of cytokine antagonism in heat stroke morbidity and mortality (Table 4). The reasons for this may be several-fold. While the injection of antibodies or protein inhibitors appears to be a straightforward method, there are several technical difficulties inherent in drug application studies (for a review, see Leon, 2005). In some cases commercial availability of an antagonist is lacking such that an experiment using this traditional technique is not feasible. It is anticipated that the increased availability of gene knockout models and more sophisticated, specific drug agents (e.g., small interfering RNAs (siRNAs)) will alleviate this experimental limitation for future studies. In other cases, the inherent properties of the cytokine and its receptors make study difficult. While in most cases effective neutralization of a cytokine can be achieved using an antibody or soluble receptor, there are instances when these substances paradoxically increase the endogenous action of the cytokine. For example, IL-6 antibodies as well as IL-6:soluble IL-6 receptor (sIL-6R) complexes have been shown to enhance, rather than limit, several endogenous actions of IL-6 (May et al., 1993; Schobitz et al., 1995; Peters et al., 1996).

Evidence supporting a role for cytokines in heat stroke pathophysiology includes: (1) increased circulating levels of cytokines in patients and experimental animals at end-stage heat stroke or in response to WBH, (2) beneficial effects of IL-1 antagonism on rat heat stroke survival (the effectiveness of antagonism of other cytokines has not been reported and IL-1 antagonism in other species has not been tested), and (3) the induction of heat stroke symptoms following cytokine injection in experimental animal models. Indirect evidence for a role of endogenous cytokines in heat stroke is suggested by the association of endotoxemia with heat stroke, the known role of cytokines in the endotoxemic syndrome, and reciprocal cytokine and heat shock protein (HSP) interactions that have been demonstrated *in vitro* in response to endotoxin treatment with or without heat stress. Each line of research will be discussed in more detail below.

Table 4. Current data supporting a role for cytokines in heat stroke

	Criterion 1: injection effects	Criterion 2: increased production	Criterion 3: neutralization
IL-1α	NT	✔	✔
IL-1β	✔	✔	✔
IL-1ra	NT	✔	NT
IL-1R1	NT	NT	✔
IL-2	NT	✔	NT
IL-2R	NT	✔	NT
IL-4	NT	✔	NT
IL-6	✔	✔	✔
sIL-6R	NT	✔	NT
IL-8	NT	✔	NT
IL-10	✔	✔	NT
IL-12p40	NT	✔	NT
IL-12p70	NT	NT	NT
IFNα	NT	✔	NT
IFNγ	NT	✔	NT
TNFα	✔	✔	NT
TNFβ	NT	NT	NT
sTNFR p55	NT	✔	NT
sTNFR p75	NT	✔	NT
TNFR p55/p75	NT	NT	✔
MIP-1α	NT	✔	NT
MCP-1	NT	✔	NT
GCSF	NT	✔	NT

Source: Adapted and used with permission from Kluger, 1991.
Note: *Criterion 1* — injected/infused cytokine induces heat stroke morbidity/mortality; *Criterion 2* — cytokine gene expression/release/production is temporally correlated with heat stroke morbidity/mortality; *Criterion 3* — cytokine neutralization inhibits or eliminates the heat stroke morbidity/mortality, NT — not tested.

Human heat stroke: correlation studies

Due to the ethical concerns of exposing human subjects to thermal extremes, the study of heat stroke responses is limited to clinical and field studies of those patients presenting with the syndrome. The annual Muslim pilgrimage to Makkah (the Hajj) has provided a rich source of data regarding cytokine changes during exertional heat stroke. While heat stroke during the Hajj has been described as the classical form (Bouchama et al., 1991, 1993), it is more representative of a mixed

type since exertional features are clearly present. This is a consequence of the variety of rituals associated with this event, many of which require significant physical exertion by the participant. As such, heat stroke etiology in this population is expected to be multi-factorial.

Located in the hot, arid desert environment of Saudi Arabia, the Hajj takes place in the extreme weather months of May to September, when temperatures range from 38 to 50°C (Khogali, 1983). These weather conditions combined with physical exertion (first day consists of a 3.5 km jog), heavy clothing that is traditional to the region (limited heat dissipation), lack of sleep (due to a rigorous schedule), and an aged population (~50 years, which is an advanced age for this region) predisposes many individuals to heat injury. The effect of clothing is a particular concern for Muslim women who are required to wear more clothing that covers a larger surface area of the body and is darker in color than that worn by men (Hashim et al., 1997). The life-threatening effects of heat stroke under these conditions are the major concern, but heat exhaustion with water or salt depletion is also prevalent. It is likely that the high occurrence of this milder form of heat illness is a result of many of the religious participants being from neighboring regions in which acclimatization to the warmer ambient conditions has occurred. However, medical conditions such as diabetes, cardiovascular abnormalities or communicable, parasitic diseases are common and predispose to heat stroke in this population (Khogali, 1983). As exemplified in the 1980s when ~2 million people participated in the Hajj, overcrowding and congestion also impose large demands on sanitation services, raising health concerns. Unfortunately, it is expected that advances in modern technologies (e.g., more rapid transport to the area) will introduce additional factors (e.g., lack of acclimatization, increased congestion, air pollution) to this complex situation.

In the late 1980s to early 1990s, several studies were conducted during the Hajj to characterize peripheral cytokine and immune disturbances in heat stroke cases. Several studies at the Hajj determined circulating cytokine concentrations at the time of clinical presentation and following cooling therapy. At the time of admission, elevations in circulating concentrations of IL-1α, IL-1β, IL-1 receptor antagonist (IL-1ra), IL-6, soluble IL-6 receptor (sIL-6R), IL-10, interferon (IFN)γ, TNFα, and soluble TNF receptors (sTNFR60 and sTNFR80) are observed (Bouchama, et al., 1991, 1993, 2000; Hammami et al., 1997; Hashim et al., 1997). In some cases, only 30–40% of patients show increased concentration of a particular cytokine (e.g., IL-1β and IL-10; Bouchama et al., 1993, 2000), whereas other cytokines, such as IL-6, are often significantly elevated in 100% of patients (Bouchama et al., 1993). IL-6 shows the highest correlation with mortality and neurologic symptoms, thus implicating it as a potential therapeutic target for heat stroke prevention/treatment strategies (Bouchama et al., 1993; Hammami et al., 1997; Hashim et al., 1997). Although attempts to correlate IL-6 with core temperature at admission have been unsuccessful, this may be related to variability in presentation times (i.e., wide core temperature range), as previously described. However, sIL-6R, sTNFR60, and sTNFR80 concentrations show a direct correlation with post-cooling in one study (Hammami et al., 1997).

An important aspect of cytokine analysis in heat stroke research is to understand the relationship of endogenous levels of a cytokine to its soluble receptor (or natural antagonists), such as exists for TNF and IL-6. It is currently unclear if antagonism of endogenous cytokine levels by their soluble receptors is directly responsible for cooling in heat stroke patients. In the study by Hammami et al. (1997), TNFα and β concentrations were undetectable at the time of clinical admission, whereas sTNFR60 and sTNFR80 concentrations were significantly elevated above controls. The inability to detect circulating TNF concentrations may be the result of localized production of the cytokine (not detectable in serum samples) or the neutralizing activity of the sTNFRs, the latter of which may interfere with assay detection of the cytokine. Interestingly, heat stroke survivors had higher sTNFR concentrations than non-survivors, suggesting a potential detrimental effect of TNF in this syndrome; unfortunately, the small sample size ($N = 3$) in this study precludes a definitive

conclusion as to the role of these receptors and endogenous TNF in human heat stroke mortality.

IL-6 has been correlated with heat stroke mortality and shows reciprocal changes with respect to its sIL-6R from time of clinical admission to post-cooling (Hammami et al., 1997). A definitive role for the sIL-6R in heat stroke pathophysiology is currently unrecognized, but two scenarios have been postulated (Hammami et al., 1997). The first scenario suggests that in the presence of high IL-6, the formation of IL-6:sIL-6R complexes may potentiate the effects of endogenous IL-6. A potentiating effect of sIL-6R on IL-6-induced responses has been previously demonstrated. In rats, the intracerebroventricular (i.c.v.) injection of sIL-6R augments and prolongs the fever and motor activity suppressing effect of IL-6 injection (Schobitz et al., 1995). These effects are mediated following its integration into cell membranes and subsequent association with the signal transducing gp130 molecule. The net effect of sIL-6R injection is an increase in the total concentration of available IL-6 signaling receptors on any particular cell type (Schobitz et al., 1995). The alternative scenario postulates that in the presence of low concentrations of IL-6 (such as occurs during cooling), sIL-6R may compete with cellular bound IL-6R for the low bioavailability of IL-6, resulting in reduced signaling. It is unclear if either of these scenarios is operating *in vivo* in the heat stroke syndrome. Future studies to examine the relationship of circulating cytokines and their soluble receptors (IL-6:sIL-R, TNF:sTNFR) or receptor antagonists (IL-1:IL-1ra) in heat stroke pathophysiology are warranted to test these hypotheses.

There is controversy in the literature regarding the most efficacious cooling method of heat stroke patients (for a review, see Hadad et al., 2004). The studies conducted at the Hajj employed evaporative cooling techniques (the most common clinical cooling therapy), thus providing insight into the effect of rapid core temperature reductions on cytokine production and clearance. Not surprisingly, rapid cooling rates are correlated with survival, cooling rates differ widely between heat stroke patients, and core temperature varies widely following cooling therapy (<38.0° to 39.4°C), which unfortunately corresponds to the time of

sample collection in some studies (Hammami et al., 1997; Hashim et al., 1997). An analysis of cytokine levels post-cooling indicates increases in sIL-6R, sTNFR80 and decreases in IL-1, IL-10, and TNFα (Bouchama et al., 1991, 2000; Hammami et al., 1997; Hashim et al., 1997). Again, high IL-6 levels are associated with mortality, as sustainment of high IL-6 levels post-cooling correlates with non-survival (Hashim et al., 1997).

The correlation of high IFNγ levels with poor prognosis in non-heat related illness has stimulated investigations into a potential role of this cytokine in the morbidity and mortality of heat stroke. Bouchama et al. (1993) showed increased serum IFNγ levels in >50% of patients presenting to the clinic with exertional heat stroke prior to cooling therapy. Elevated circulating levels of IL-1β and IL-6 were also observed, although these samples were obtained in a different set of patients than those in which IFNγ was detected. Whereas IL-6 levels tend to be highest in non-survivors, IFNγ levels do not correlate as strongly with heat stroke morbidity/mortality (Bouchama et al., 1993). Sonna et al. (2004) hypothesized prodromal viral illness as the stimulus for increased expression of interferon-inducible (IFI) genes observed in peripheral blood mononuclear cells (PBMC) from military recruits that collapsed from exertional heat stroke during basic training. It is difficult to speculate from this study as to the incidence of the combination of viral illness with heat stroke in this population due to the small number of screened subjects ($N = 4$). However, it does provide food-for-thought regarding the appropriateness of pre-screening individuals for illness and/or elevated cytokine levels prior to heat exposure as a potential protective measure against injury or death.

In military recruits with exertional heat stroke, plasma levels of IFNγ are elevated concomitantly with IL-1β, IL-6, TNFα, IL-2 receptor (IL-2R), and IL-8; elevated IFNγ, IL-6, and IL-2R levels correlate with morbidity in this study (Lu et al., 2004). Differences between studies in the correlation of IFNγ with mortality (e.g., Bouchama et al., 1993; Lu et al., 2004) may be due to several factors. First, military recruits in the study by Lu et al. (2004) participated in a well-defined exercise regimen whereas the specific rituals experienced by

participants of the Hajj (Bouchama et al., 1993) are not as well documented for each exertional heat stroke case. Second, circulating cytokine levels in heat stroke patients were compared to normal controls in one study (Bouchama et al., 2000) and to exercising controls that did not experience heat stroke symptoms in the other (Lu et al., 2004). A complicating factor in many studies of this nature is the control population that is used for comparison of cytokine responses to the heat stroke condition. For those studies conducted at the Hajj, it is a logistical impossibility to obtain plasma samples from a group of individuals that represent an appropriate control population. In many cases, comparisons to a control population are not provided; rather, pre- (time of clinical admission) and post-cooling (8 or 24 h after cooling) values are directly compared to one another with, in most cases, further characterization provided between survivors and non-survivors (Bouchama et al., 1991; Hashim et al., 1997). Other studies have compared heat stroke values to those observed in a control "normal" population (typically undefined, but presumably at rest; Bouchama et al., 1993) or, more appropriately, to individuals participating in the same event with heat exposure, but in the absence of heat stroke (Bouchama et al., 2000; Lu et al., 2004). A comparison of heat stroke values to both heat exposed and normothermic controls in the resting and exercise condition is the most appropriate scientific design, but is achieved in few studies. Studies on military populations are typically more controlled, but also present with inherent difficulties since non-exercising controls are typically not available at the time of heat stroke collapse. As such, these difficulties speak to the importance of animal experimentation for an understanding of the complex etiology of exertional heat stroke since the multitude of factors inherent in this syndrome can be more easily controlled in the laboratory setting. However, as will be described in more detail below, animal models of passive, rather than exertional heat stroke (the latter being more representative of the human population) are typically used to explore cytokine responses *in vivo*.

Several attempts have been made to correlate cytokine changes with different aspects of the heat stroke syndrome, such as hyperthermia, immune

disturbances, and heat severity. It is typically difficult to find a strong correlation between cytokine levels and core temperature in human heat stroke cases, due to differences in clinical treatment strategies and presentation times. For example, weak correlations between reported core temperature and cytokine values are common as patients are subjected to different cooling regimens and durations (Bouchama et al., 1991, 1993, 2000; Hammami et al., 1997; Hashim et al., 1997; Sonna et al., 2004). However, in one study, the ability to cool patients from 40° to 38°C was dependent on serum IL-1β (Chang, 1993). This is the only report implicating endogenous IL-1β in the control of core temperature responses in heat stroke patients. This seems rather surprising since several of the cytokines implicated in heat stroke pathophysiology are known regulators of core temperature in health and disease. On the other hand, the inability to correlate circulating cytokine concentrations with heat-induced core temperature changes may be due to tissue concentrations being more important than circulating levels in the mediation of these responses.

Several studies have attempted to correlate changes in circulating cytokine concentrations with different aspects of the heat-induced acute phase response (APR). IL-6 modulates the APR to infection/inflammation (such as that induced by endotoxin) and is known to stimulate hepatic synthesis of C-reactive protein (CRP, a sensitive marker of inflammation; Heinrich et al., 1990). Hashim et al. (1997) reported elevated CRP levels in survivors and non-survivors of exertional heat stroke, but were unable to correlate these changes with elevations in IL-6. Elevated ALT levels suggested potential hepatic dysfunction in these patients, but did not correlate with CRP. LPS is a cell wall component of gram-negative bacteria that increases in concentration in human heat stroke cases, although this response does not show a direct correlation with circulating levels of IL-1 or TNFα (Bouchama et al., 1991). The two hypotheses postulated to account for these findings include having missed the time of peak cytokine levels in some patients as presentation times varied so widely, and the greater importance of tissue vs. circulating cytokine concentrations in these

analyses (Bouchama et al., 1991). Although several additional pathophysiological responses have been observed, including hyper- and hypoglycemia, lactic acidosis, and leukocytosis, potential correlations between cytokine levels and these responses have not been examined. Attempts to correlate elevated cytokine levels with one another have also provided contradictory results. IL-1 and TNFα levels did not correlate with one another in one study, whereas significant correlations between IL-1β, TNFα, and IL-6 have been observed (Chang, 1993). The reason(s) for these discrepant results is not immediately evident.

Animal models of heat stroke

While several animal models have been developed for the study of pathophysiologic responses to heat stroke (Adolph, 1947; Hubbard et al., 1976, 1977; Wright, 1976; Wright et al., 1977; Dubose et al., 1983a; Lord et al., 1984; Gathiram et al., 1987a; Wilkinson et al., 1988; Romanovsky and Blatteis, 1996; Hall et al., 2001), few studies have examined the role of cytokines. Elevated circulating concentrations of IL-1, IL-6, IL-8, IL-10, TNF, and granulocyte colony stimulating factor have been reported with localized or WBH in primates (Bouchama et al., 2005), rabbits (Lin et al., 1994), mice (Neville and Sauder, 1988), and rats (Chiu et al., 1995, 1996; Haveman et al., 1996; Lin et al., 1997; Liu et al., 2000). As shown in human heat stroke, IL-6 is correlated with heat stroke severity in a primate model of passive heat stroke (Bouchama et al., 2005).

Unfortunately, most animal studies have determined cytokine concentrations at maximum heat stress, thus ignoring changes in the production of these mediators throughout exposure *and recovery*. Furthermore, cytokine determinations have typically been performed on plasma/serum samples — the relation of these measurements to cytokine changes occurring at the tissue level (i.e., potential site(s) of heat injury) are unknown. Recently, my laboratory characterized the plasma and tissue (liver) profile of 11 cytokines (IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12p40, IL-12p70, IFNγ, MIP-1α, TNFα) at maximum heat stress and

throughout 24 h of recovery in a mouse model of passive heat exposure. The goal of this experiment was to correlate cytokine changes with the profound thermoregulatory responses (hypothermia and fever) observed during recovery. As shown in Fig. 6, only plasma IL-12p40 was elevated at maximum core temperature (CTM of 42.7°C in this model; Leon et al., 2005, 2006). On the other hand, hypothermia was associated with significantly elevated concentrations of IL-1β, IL-6, and IL-10; it is unknown if these cytokines are regulating this core temperature response or are a consequence of low core temperature, since hypothermia has been shown to exacerbate cytokine production in other models (Arons et al., 1999; Fairchild et al., 2004). Heat-induced hypothermia was also associated with increased levels of IL-1α in the liver, despite an inability to detect this cytokine in the plasma (Fig. 7); as previously described, these data suggest a potential dissociation between cytokine levels measured in the blood and those directly related to organ (dys)function. Interestingly, at 24 h after the start of heat exposure (~0900–1000 h the following day), mice showed a fever-like core temperature response (~1.0° to 1.5°C above controls) that correlated with significantly increased levels of IL-6, a known endogenous pyrogen (Fig. 6; Leon et al., 2006). At present, it is unclear if IL-6 is modulating this core temperature response, which again speaks to the importance of investigating the effectiveness of cytokine antagonism/neutralization in altering core temperature responses and mortality in the heat syndrome. Importantly, similarities in cytokine responses between passive (animal) and exertional (human) cases of heat stroke are supportive of the appropriateness of animal models to address this issue, although exertional animal models need to be developed.

IL-1 and heat stroke

Lin et al. (1994) reported increased plasma and hypothalamic levels of IL-1β in heat stroked rabbits. IL-1 levels in other brain areas, including the medulla oblongata, cortex, and spinal cord, were unaffected. The anterior hypothalamus is thought
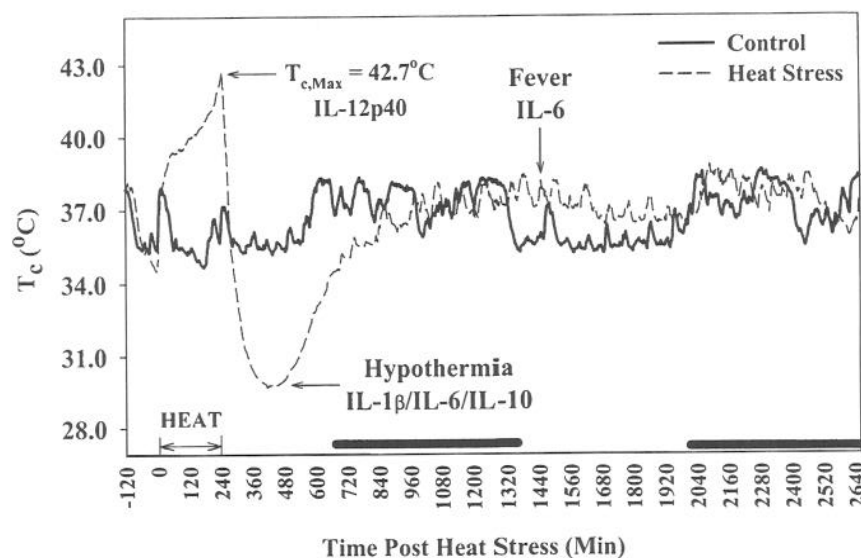
Fig. 6. Summary of plasma cytokine changes observed in male C57BL/6J mice during 48 h of heat stress recovery. Details of the heat stress protocol and cytokine findings are described elsewhere (Leon et al., 2005, 2006). Mice were heat stressed to a maximum core temperature of 42.7°C and then allowed to recover at an ambient temperature of 25 ± 2°C until sample collection. Mice were sacrificed at each time point for tissue collection ($N = 8$–11 mice/group). Core temperature response is depicted for one representative control and heat stressed mouse. Note that IL-12p40 was the only cytokine significantly elevated at $T_{c,Max}$ (42.7°C). The largest cytokine changes were observed at hypothermia, where IL-1β, IL-6, and IL-10 were significantly elevated. IL-6 was the only cytokine that remained elevated 24 h following heat stress, which is the time point at which mice displayed a fever-like (~1.0° to 1.5°C) core temperature elevation. Note that IL-1α, IL-2, IL-4, IL-12p70, IFNγ, MIP-1α, and TNFα were not detectable in the plasma at any time point. Black horizontal bars represent lights-off period in a 12:12 h L:D cycle. Core temperature was collected at 1 min intervals in conscious, freely moving mice using intraperitoneally implanted radiotelemetry devices (Data Sciences International, St. Paul, MN). Cytokine determinations were performed on duplicate samples using the FlowMetrix™ System (Luminex, Austin, TX), which permits the simultaneous quantitation of multiple cytokines (Leon et al., 2006).

to be the main integration site of thermoregulatory responses. While systemic injection of the IL-1ra (a naturally occurring antagonist of endogenous IL-1 actions) attenuated the rectal temperature response to heat stroke, the effectiveness of this treatment following administration directly into the hypothalamus was not tested (Lin et al., 1994). In addition, it is unclear if attenuation of the hyperthermic response was an indirect response due to a reduction in cardiovascular strain or vice versa; thus, the specific role of endogenous IL-1β (and other cytokines) on thermoregulatory control mechanisms during heat exposure and/or recovery remains unknown. Unfortunately, control levels of IL-1β were also elevated in this study in response to anesthesia. Anesthesia is commonly used in animal experimentation and represents a confounding factor due to its known effects on thermoregulatory control processes and cytokine

production (Stoen and Sessler, 1990; Hanagata et al., 1995; Brix-Christensen et al., 1998). It is also unclear if increased heat-induced brain levels of IL-1β were due to local production by hypothalamic tissue or systemic production and infiltration into hypothalamic tissue after IL-1β had traversed the blood brain barrier (BBB; Lin et al., 1994).

Increased hypothalamic IL-1β levels have been implicated in heat-induced cerebral ischemia in animal models (Lin et al., 1994). A connection between heat stroke, IL-1β and central concentrations of dopamine (DA) and serotonin (5-HT) may account for this response. Sharma et al. (1994) demonstrated a protective effect of the selective 5-HT₂-receptor antagonist ketanserin as cerebral edema was reduced following a 4 h heat exposure in the rat. Selective brain depletion of 5-HT following i.c.v. administration of
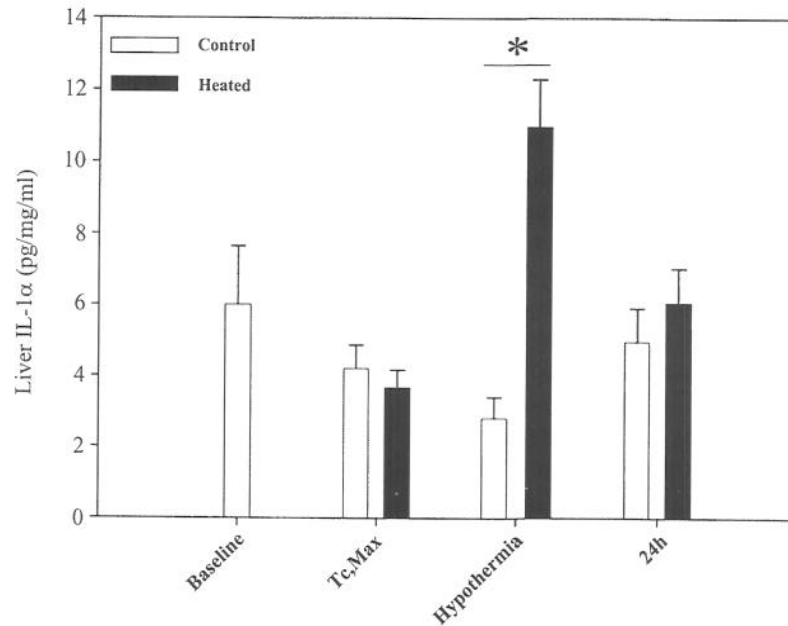
Fig. 7. Liver IL-1α levels in control and heat stressed male C57BL/6J mice. Mice were heat stressed to a maximum core temperature of 42.7°C and allowed to recover at an ambient temperature of 25±2°C until sample collection. Mice were sacrificed at each time point for tissue collection ($N = 4$–7 mice/group). IL-1α was the only cytokine that was detectable at an increased level in the liver of heat stressed mice compared to controls. IL-1α was significantly elevated in heat stressed mice at hypothermia; note that increased plasma levels of IL-1α were not detectable at any time point in heat stressed mice. Cytokine levels were normalized to tissue protein concentration (~50 mg/ml of sample). Cytokine determinations were performed on duplicate samples using the FlowMetrix System (Luminex, Austin, TX), which permits the simultaneous quantitation of multiple cytokines. *Indicates significance at $P < 0.05$.

5,7-dihydroxytryptamine (5,7-DHT) also showed protection in heat-stroked rats, as indicated by significant reductions in arterial hypotension, cerebral ischemia, hypothalamic 5-HT accumulation, cerebral neuronal damage, and prolonged heat stroke survival time (Kao and Lin, 1996). The mechanism of 5-HT effects in the induction of cerebral edema is thought to be due to a reduction in mean arterial pressure (MAP) and an increase in intracerebral pressure, which inhibits cerebral flow resulting in neuronal injury (Kao and Lin, 1996). Similarly, intravenous (i.v.) administration of the IL-1ra prolonged survival time and attenuated arterial hypotension and hypothalamic 5-HT accumulation (Chiu et al., 1995). The ability of intrahypothalamically injected IL-1β to induce 5-HT production has also been demonstrated in a rat model (Shintani et al., 1993), and IL-1 and 5-HT have been implicated in the regulation of BBB permeability (Sharma and Dey, 1986, 1987).

Together, these studies support a central action of 5-HT and IL-1 in the mediation of heat injury and cerebral ischemia. The potential role of other endogenous cytokines in these 5-HT-mediated events is currently unknown.

Increased striatal DA concentrations are correlated with cerebral ischemia in the rat (Kao et al., 1994a, b; Chiu et al., 1996) and destruction of the nigrostriatal dopaminergic system following i.c.v. injection with 6-hydroxydopamine (6-OHDA) increases striatal blood flow, reduces ischemic damage, and prolongs survival time in rats (Lin et al., 1995). A connection between IL-1 and DA is suggested by the correlation between prolonged heat stroke survival and attenuation of striatal DA release in rats treated with the IL-1ra (Chiu et al., 1996); the ability of IL-1β to increase hypothalamic levels of DA has also been reported (Shintani et al., 1993). DA also appears to modulate the HPA axis response to heat-induced dehydration, as the D_2

receptor antagonist haloperidol attenuates cortisol release in humans (Hennig et al., 1995). Glucocorticoid hormones (corticosterone in rodents and cortisol in humans) are produced by the adrenal gland and represent an integral component of the HPA axis. In addition to the mobilization of energy stores during stress, glucocorticoids inhibit cytokine production in response to stress (Waage et al., 1990; Barber et al., 1993; Di Santo et al., 1996). Classical heat stress has been shown to induce cortisol secretion with heat stroke patients showing up to a five-fold elevation of plasma concentrations (Collins et al., 1969; Follenius et al., 1982; Laatikanen et al., 1988; Al-Harthi et al., 1990). Glucocorticoids are commonly used prophylactically, or their production is inhibited by adrenalectomy (ADX), to examine changes in cytokine and core temperature responses to environmental stimuli in experimental animals. Liu et al. (2000) treated rats with the synthetic glucocorticoid dexamethasone and showed an attenuation of heat stroke-induced hypotension, cerebral ischemia, neuronal damage, and prolongation to time of death; the protective effect of dexamethasone treatment was not due to a reduction in heat-induced core temperature. In the same study, the removal of endogenous glucocorticoids by ADX increased susceptibility of rats to heat stroke — an effect that was reversed by dexamethasone replacement therapy (Liu et al., 2000). Unfortunately, ADX rats were not provided replacement baseline levels of glucocorticoids in this study, which may have altered the observed responses. For example, the exacerbated arterial hypotension observed in ADX rats may have resulted from the absence of the normal permissive action of glucocorticoids on the cardiovascular response to catecholamines and other hormones that are released by heat exposure (Liu et al., 2000). However, the effects of glucocorticoids were correlated with their ability to decrease plasma IL-1β levels at the onset of heat stroke, implicating cytokine antagonism as the mechanism of glucocorticoid protective effects.

Cytokine injection induces heat stroke symptoms

Systemic injection of IL-1 induces many heat stroke symptoms including hypotension, decreased systemic vascular resistance, depressed myocardial function, and tissue necrosis (for review, see Dinarello, 1991). Prostanoid synthesis has been proposed as a mechanism of many IL-1 induced effects. In rabbits, cyclooxygenase inhibitors mitigate the hypotensive effects to IL-1 alone or in combination with TNF (Okusawa et al., 1988). As previously described, the ability of the IL-1ra to reduce many of the hemodynamic effects of IL-1 has directly implicated this cytokine in the mediation of these effects in heat stroke (Chiu et al., 1995, 1996; Lin et al., 1997). IL-1 acts synergistically with other cytokines, such as IL-6 and TNF, in the mediation of hemodynamic shock and tissue damage (Dinarello, 1991). The peripheral injection of IL-1β, IL-6, IL-10, and TNF induces many of the pathophysiological responses observed in human and animal heat stroke studies, including hypothermia, fever, HPA activation, increased vascular permeability, hemodynamic shock, and death (Kluger, 1991; Oshima et al., 2001; Leon, 2002, 2004; Kuwagata et al., 2003; Wieczorek et al., 2005).

Cytokine-mediated heat stroke responses: results from gene knockout models

As previously discussed, the study of cytokine-mediated heat stroke responses has been limited by technical difficulties, such as a lack of commercial availability of antibodies and/or antagonists. The recent advent of gene knockout technology provides a technique by which the efficacy of cytokine neutralization can be examined *in vivo*. Gene knockout mice are genetically engineered to lack a functional gene in every tissue of the body and essentially function as "chronic protein neutralization systems" (Sigmund, 1993). There is wide commercial availability of cytokine and cytokine receptor knockout mice, many of which have been used for the study of infectious and inflammatory responses. While the development of functional redundancy is an important concern in physiological research (i.e., the redundant/pleiotropic properties of cytokines may allow developmental redundancy to compensate for a missing gene's action), these models also provide several

methodological advantages over traditional techniques (reviewed in Leon, 2005).

Although high IL-6 levels are typically associated with mortality in heat stroke patients and animal models (Hashim et al., 1997; Bouchama et al., 2005), protective effects of IL-6 neutralization have not been experimentally examined. Recent data from my laboratory demonstrate *decreased* heat stress survival in IL-6 knockout mice compared to wild-type controls (33 vs. 100% survival, respectively; $P = 0.025$) with the maximum decrease occurring within 24 h of heat stress recovery (Fig. 8). These data are suggestive of a dual role for endogenous IL-6 in heat stroke responses with exacerbated (pathophysiological) levels being detrimental (as suggested by correlation studies; this has not been experimentally verified), but baseline (permissive) levels required for heat stroke survival (as suggested by results of Fig. 8). Similar results are reported for IL-6 knockout mice in the endotoxemic syndrome (Leon et al., 1998). As shown in Fig. 8B, preliminary data from TNF p55/p75 receptor (TNFR) knockout mice are equally contradictory to the findings from correlation studies. Although high TNF levels are implicated as harmful in the heat stroke condition, TNFR knockout mice (mice that produce endogenous TNF, but are unable to respond to the cytokine due to an absence of the signaling receptors) showed a tendency toward decreased survival compared to wild-type controls (40 vs. 100% survival, respectively; $P = 0.10$; note small sample size). Although future studies using traditional methods of cytokine neutralization are important to verify the findings, these data suggest that the results obtained from correlation studies may have been overstated with regards to the deleterious role of endogenous cytokines in the heat stroke syndrome.

GI permeability and endotoxemia with heat stroke

The primary cardiovascular response to heat exposure is an increase in skin blood flow to promote heat loss and reduce the rate of heat gain from the environment. Increased skin blood flow is accompanied by a fall in splanchnic blood flow as a compensatory mechanism to maintain blood pressure. A severe reduction of intestinal blood flow results in GI ischemia and increased vascular permeability — the latter response facilitates the leakage of endogenous bacteria (and its toxic cell wall component, LPS) into the systemic circulation, resulting in endotoxemia. While under normal conditions the liver serves as an adequate clearance organ for endotoxin, thermal injury to this organ may compromise its clearance function, permitting endotoxin leakage into the portal circulation. It has been postulated that increased cytokine expression is a direct consequence of endotoxin leakage under heat stroke conditions (Fig. 9).

GI permeability and heat stroke

Physical and physiological disruptions of GI barrier function facilitate luminal translocation of intestinal contents to the blood. Endothelial barrier dysfunction occurs in response to a variety of stimuli including endotoxemia (Unno et al., 1997), trauma/hemorrhage (Roumen et al., 1993), strenuous exercise (Brock-Utne et al., 1988; Pals et al., 1997), and hyperthermia/heat stroke (Wijsman and Shivers, 1993; Moseley et al., 1994; Hall et al., 2001). With heat exposure, increased endothelial permeability is commonly observed in the GI tract and the BBB (Wijsman and Shivers, 1993; Moseley et al., 1994; Lambert et al., 2002, 2004). A clinical indicator of GI barrier disruption is detectable levels of endotoxin in the portal or systemic circulation.

Compensatory splanchnic vascular responses to heat strain cause a reduction in intestinal blood flow and the promotion of intestinal oxidative and nitrosative stress, which compromises GI permeability (Hall et al., 2001; Lambert, 2004). Nitric oxide (NO; otherwise known as endothelium-derived relaxing factor) is one of several free radicals whose production increases with hyperthermia. Increased NO concentrations are detectable in peripheral splanchnic vascular beds of hyperthermic animals and are thought to increase tight junction permeability (Hall et al., 1994). It is thought that constitutive NO synthesis provides a protective function
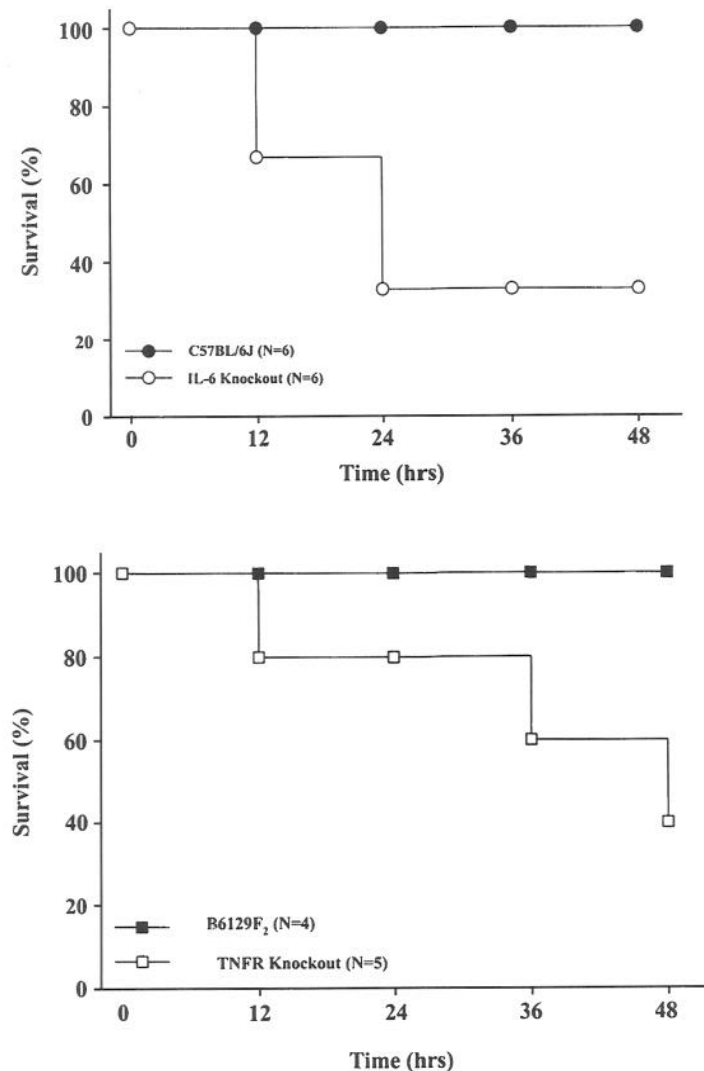
Fig. 8. Forty-eight hour survival curves of heat stressed male IL-6 (top) and TNF p55/p75 receptor (TNFR) knockout mice (bottom) compared to their respective controls. Details of the heat stress protocol have been described in detail elsewhere (Leon et al., 2005). Mice were heat stressed to a maximum core temperature of 42.7°C and allowed to recover, undisturbed, for 48 h at an ambient temperature of 25 ± 2°C. Survival was assessed visually and through inspection of radiotelemetry measurements every 12 h. Note that IL-6 and TNFR knockout mice showed significantly decreased survival rates compared to controls, despite high serum IL-6 and TNF levels being correlated with heat stroke mortality in human and animal models (see text for details).

under hyperthermic conditions by buffering increases in splanchnic vasoconstrictor activity and cellular stress (Hall et al., 2001). Increased intestinal barrier permeability occurs following cytokine-induced stimulation of NOS II enzymatic activity, which increases cellular NO flux above constitutive levels; the resultant overproduction of NO inhibits splanchnic resistance, leading to hypotension and

circulatory collapse with heat stroke. Findings of lowered heat tolerance and increased venous portal endotoxin levels in heat stressed rats treated with the nitric oxide synthase inhibitor, L-NAME, support this hypothesis. Increased microvascular permeability has also been reported in cats, under non-heat stress conditions, following L-NAME treatment (Kubes and Granger, 1992). An increase in
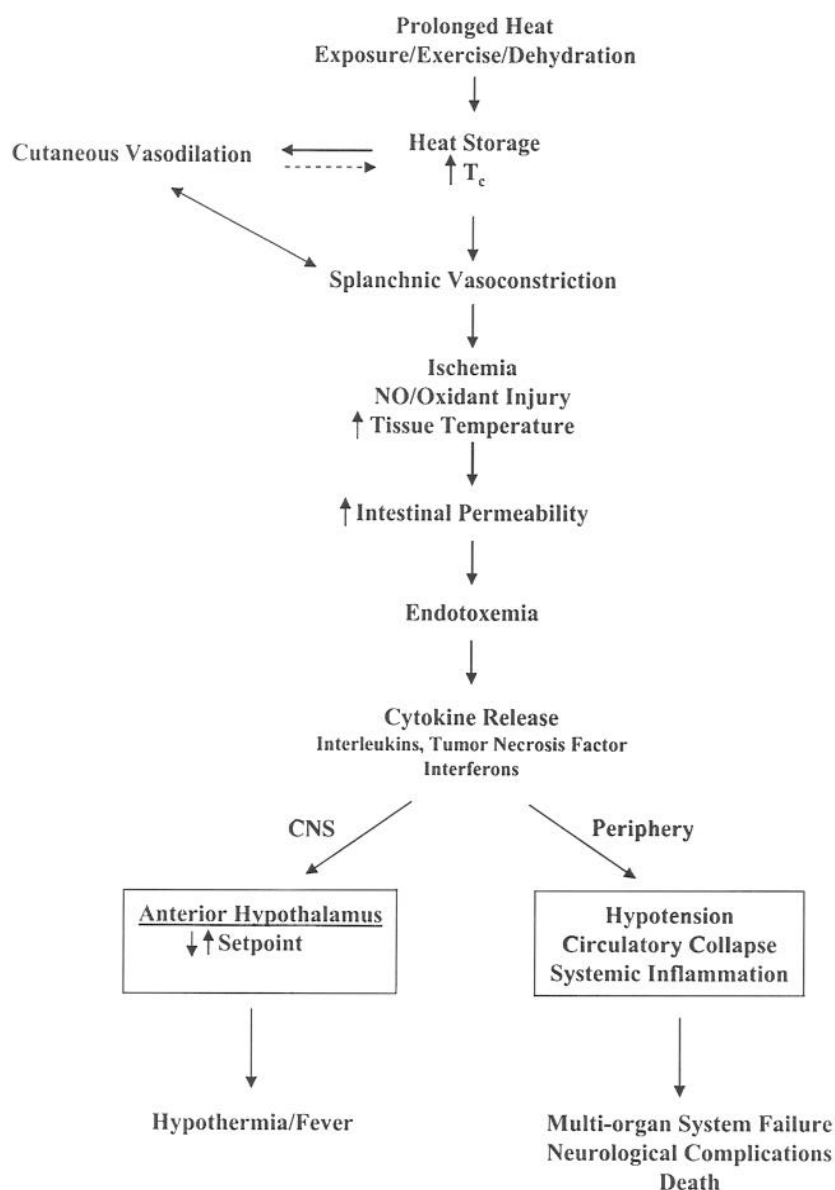
Fig. 9. Effect of prolonged heat exposure on changes in blood flow, intestinal permeability, and cytokine release. Heat exposure induces a rise in core temperature (T_c), which stimulates an increase in cutaneous blood flow to facilitate heat dissipation to the environment. Splanchnic vasoconstriction represents a compensatory response that is aimed at facilitating cutaneous vasodilation. The resultant ischemia of the intestines causes oxidative and nitrosative stress, which results in an increase in gut permeability. As intestinal membrane permeability is increased, endotoxin leakage is enhanced and stimulates cytokine (e.g., IL-1, IL-6, IL-10) production. Increased cytokine production signals to the periphery and CNS, which may induce changes in core temperature (fever, hypothermia) and multi-organ system failure (the ultimate cause of heat stroke mortality).

leukocyte adhesion is the proposed mechanism of L-NAME effects, as NO is a known modulator of leukocyte adherence (Kubes et al., 1991; Kubes and Granger, 1992).

Inhibition of leukocyte recruitment is also a proposed mechanism for the protective effects of IL-10 on alterations of vascular permeability with endotoxemia. Hickey et al. (1998) reported greater

leukocyte recruitment and vascular permeability of IL-10 knockout mice compared to wild-type controls at 4 h following peripheral injection of LPS. The combined blockade of E- and P-selectin reduced leukocyte rolling to control levels in IL-10 knockout mice, suggesting a direct effect of IL-10 on the expression of these adhesion molecules (Hickey et al., 1998). IL-10 is a Th2 type cytokine that has potent inhibitory actions on the production of pro-inflammatory cytokines, such as IFNγ, IL-1, IL-6, and TNF. In a model of delayed-type hypersensitivity in mice, IL-10 treatment significantly inhibited footpad swelling induced by injection of Th1 clones and reduced local footpad levels of IL-2, IL-6, IFNγ, and TNF (Li et al., 1994). IL-10 is a known inhibitor of contact hypersensitivity — Berg et al. (1995) examined the extent of ear swelling in response to croton oil (a skin irritant) and showed twice the amount of swelling in IL-10 knockout mice compared to their wild-type controls. Swelling persisted through 48 h in the knockout mice, whereas it showed ∼50% abatement at this time point in wild-type mice. Treatment with an anti-TNF antibody reduced tissue necrosis in IL-10 knockout mice and eliminated focal necrosis in wild-type mice; thus, negative regulation of TNF production is a proposed permeability control mechanism mediated by IL-10 (Berg et al., 1995).

Additional cytokine interactions in the control of intestinal permeability include IL-10 modulation of IFNγ and IL-6 production. In a cecal ligation and puncture (CLP) model of sepsis, wild-type mice showed significantly increased IL-6 levels in the ileal mucosa which correlated with increased intestinal permeability (Wang et al., 2001a). In IL-6 knockout mice, CLP was without effect on intestinal permeability, presumably due to ∼20-fold higher mucosal level of IL-10 in these animals. The occurrence of increased intestinal permeability in wild-type mice, despite increased IL-10 levels (albeit below the level observed in the knockout animals) is suggestive of the importance of understanding the ratio of IL-6 to IL-10 levels in the mediation of this response in sepsis (Wang et al., 2001a). Finally, IFN-γ has been implicated in endothelial barrier dysfunction due to its ability to enhance expression of endothelial cell adhesion molecules — this effect being mediated via the stimulated production of other cytokines (TNFα, IL-1β) and free oxygen radicals (e.g., NO; Ruszczak et al., 1990). IL-10 pretreatment has been shown to prevent IFN-γ-induced increases in vascular permeability in human umbilical vein endothelial cells (Oshima et al., 2001).

Endotoxemia and heat stroke

In exertional heat stroke patients, TNFα, IL-1α, and endotoxin were detectable at clinical admission (core temperature ∼42.1°C), and showed a significant decrease following the completion of cooling (Bouchama et al., 1991). Changes in LPS concentrations occurred independently from cytokine production and the decrease in core temperature with cooling. High endotoxin levels were detected in a young athlete (core temperature 40.6°C) on the second day of football practice and may have been related to hemorrhagic necrosis of the liver (Graber et al., 1971). Conversely, Chung et al. (1999) were unable to measure plasma endotoxin in former heat stroke patients or controls that were exposed to a 60 min heat stress (core temperature <39.5°C). Presumably, this negative response was related to the relatively low core temperature increase induced by the 1 h heat exposure; in primates, elevations in circulating endotoxin were detectable as core temperature approached 41.5°C and then showed a precipitous increase starting at ∼43.0°C (Gathiram et al., 1987a). It is noteworthy that splanchnic blood flow shows an initial decrease at core temperature of 40°C (Hall et al., 2001) and liver damage is typically detectable at core temperatures of ∼42° to 43°C (Kew et al., 1970; Bowers et al., 1978; Chao et al., 1981).

Several methods have been used to explore the role of endotoxin in heat stroke responses. Bynum et al. (1979) demonstrated increased survival to experimental heat stroke in the dog following a reduction of gut flora by antibiotics — 18 h survival increased more than three-fold, as long as treatment was provided prior to heat exposure. Similarly, the rise in core temperature and incidence of endotoxemia in rabbits was reduced following oral antibiotics (Butkow et al., 1984). In primates, 24 h

pretreatment with anti-LPS hyper-immune serum returned plasma LPS levels to baseline and reversed mortality (Gathiram et al., 1987b). However, this protective effect was inhibited following heating of the animals to a higher final core temperature, indicating that hyperthermia alone may cause irreversible organ damage and death (Gathiram et al., 1987b); interestingly, mortality was observed following only ~0.3°C further increase in core temperature (43.5 vs. 43.8°C). Dubose et al. (1983a) reported similar findings in endotoxin-tolerant rats which were protected from heat stroke mortality under moderate heat stress conditions — this protective effect dissipated once the accumulated thermal load (calculated as thermal area) exceeded a threshold value of > 60°C/min (referred to as severe heat stress). Interestingly, an increase in endotoxin sensitivity following zymosan (complement antagonist) injection had no effect on thermotolerance (Dubose et al., 1983a). Furthermore, increased endotoxin concentrations were undetectable in plasma or tissues of heat stressed rats in this study. The authors concluded from these findings that the protective effect of endotoxin tolerance may have been unrelated to heat-induced endotoxemia *per se*, but rather a generalized protective response against the shock-like (SIRS) syndrome induced by heat — similar to that observed in response to hemorrhage or trauma (Dubose, et al., 1983a). A subsequent study by this same laboratory suggested that the protective effect of endotoxin tolerance was related to enhanced stimulation of the reticuloendothelial (RES) system, a major route of endotoxin clearance (Dubose et al., 1983b). As such, RES stimulation reduced and RES blockade increased mortality of heat stressed rats (Dubose et al., 1983b).

While the results from the aforementioned studies support a role for multiple cytokine interactions in the maintenance of epithelial barrier integrity, an examination into their role in the vascular permeability changes that occur in the heat stroke syndrome has not yet been performed.

Heat shock proteins

Heat shock proteins are phylogenetically conserved proteins that function as molecular chaperones to prevent the misfolding and aggregation of proteins under stressful conditions (Lindquist and Craig, 1988; Fink, 1999; Jaattela, 1999; Hartl and Hayer-Hartl, 2002). Based on these functions, HSPs have traditionally been regarded as intracellular proteins; however, the presence of HSPs in the circulation of normal individuals and increased expression in response to a variety of stressful stimuli has stimulated interest in their extracellular functions (Lindquist and Craig, 1988; Jaattela, 1999; Pockley et al., 1999; Basu et al., 2000). HSPs are found in all organisms that have been examined, from bacteria to humans, and are thought to have a major role in providing cytoprotection in the face of exposure to environmental (heavy metals, heat stress), physiological (cell differentiation, protein translation), and pathological (infections, ischemia/reperfusion) stimuli (Lindquist and Craig, 1988; Jaattela, 1999; Kregel, 2002). A decrease in HSP expression with aging and increased expression under pathophysiological conditions such as hypertension and atherosclerosis suggest that these molecules may be important biomarkers of stress susceptibility (Pockley et al., 2000; Rea et al., 2001; Xu, 2002).

HSPs range in size from 27,000 to 110,000 Da and are grouped into families according to their molecular mass, cellular localization, and function. HSP 27 is a constitutively expressed protein that resides in the cell cytoplasm, but undergoes increased expression and translocation to the cell nucleus in response to heat exposure (Arrigo et al., 1988). The function of HSP 27 is thought to reside in its ability to stabilize cytoskeletal protein organization under stressful conditions (Lavoie et al., 1993). The HSP 60 family consists of mitochondrial and cytosolic members that function as molecular chaperones to facilitate protein folding (Bukau and Horwich, 1998). It has been suggested that HSPs of the 60-kDa family function as danger signals to the innate immune system, inducing the release of several cytokines implicated in chronic inflammatory conditions, as well as atherosclerosis (Kaufmann, 1990; Nomoto and Yoshikai, 1991). The ability of HSPs to interact with pattern recognition receptors, such as the LPS receptors known as CD14 and Toll, to induce cytokine release suggests that interactions between HSPs and the host immune system may be an important first line of defense against

infection/inflammation. The HSP 70 family consists of constitutive cytosolic HSP 73 (also known as HSC 70), stress-inducible cytosolic HSP 72, endoplasmic reticulum Bip (also known as Grp78), and mitochondrial HSP 70 (Lindquist and Craig, 1988; Fink, 1999; Hartl and Hayer-Hartl, 2002). Proteins of the HSP 70 family have been extensively studied for their protective function(s) against a variety of stressful insults, including thermal stress (Yang et al., 1998; Li et al., 2001; Kelty et al., 2002), ischemia/reperfusion (Marber et al., 1995; Stojadinovic et al., 1995; Rajdev et al, 2000), tissue injury (Brown et al., 1989), metabolic stress such as glucose deprivation (Williams et al., 1993), and sepsis (Hotchkiss et al., 1993; Villar et al., 1994). HSP 70s are also known to function in concert with other molecular chaperones, such as HSP 90 and HSP 110. The HSP 90 family consists of cytosolic HSP 90 and glucose regulation protein (GRP) 96, the latter of which is upregulated in the ER in response to glucose starvation (Kabakov et al., 1990; Morita et al., 2000). HSP 90 has been shown to play a role in glucocorticoid receptor (GR) functioning, through facilitation of the folding of the receptor's hormone binding domain, receptor intracellular trafficking, and stabilization of the receptor against proteolytic degradation (Pratt et al., 1999). HSP 70 is thought to facilitate GR–HSP 90 heterocomplex formation and has also been shown to participate in HSP 70–HSP 90–LPS interactions (Hutchison et al., 1994). HSP 110 is a molecular chaperone that has been strongly implicated in anti-tumor immune responses. Increased HSP 110 expression has been observed in hepatocellular carcinoma and human colorectal cancer (Hwang et al., 2003; Gotoh et al., 2004) and has been hypothesized to function as a "danger signal" by stimulating antigen presenting cells (e.g., dendritic cells) to release pro-inflammatory cytokines, thus alerting the immune system to tumors (Manjili et al., 2005).

HSPs and protein folding

In addition to their protective functions in stressful environments, HSP 70 family members also interact transiently with a variety of cellular proteins to facilitate natural protein folding and maturation during normal growth and physiological functioning. Beckmann et al. (1990) suggested that most proteins interact with HSP 72 and HSP 73 during synthesis (emergence from the ribosome) as a mechanism of maintaining a stable conformation during the translation process; upon release of the HSPs, each newly synthesized protein folds into its final conformational state. Presumably, the affinity of nascent protein–HSP interactions is altered as the protein folds into its mature conformation and neighboring peptide domains interact with one another, thus releasing HSP in an ATP-dependent process (Beckmann et al., 1990). There are several lines of evidence to suggest that HSP 70s exist in an equilibrium state between free and substrate-bound forms; that is, as free HSP is reduced following binding to newly synthesized proteins, additional HSP synthesis occurs. When the protein folds into its mature conformation and releases HSP 70, the free pool is increased once again and new HSP synthesis is halted. Evidence to support this type of regulation includes: (1) HSP 70 synthesis is increased in direct proportion to heat stress severity (and protein denaturation); (2) HSP 70 synthesis following a second stressor is influenced by the amount of pre-existing HSP 70 induced by the initial stressor; (3) HSP 70 synthesis is activated following the microinjection of denatured proteins into frog oocytes — this response may be directly related to the binding of HSP 70 to the newly injected denatured proteins, which subsequently reduces the free pool of HSP 70; (4) an immediate induction of HSP 70 synthesis occurs following the injection of anti-HSP 70 antibodies; and (5) the injection of puromycin, which stops protein translation prior to the completion of synthesis, induces increased HSP 70 synthesis (Ananthan et al., 1986; Mizzen and Welch, 1988; Beckmann et al., 1990). Taken together, these results suggest that HSP 70s transiently function as molecular chaperones to facilitate proper folding of newly synthesized proteins.

Alterations in HSP expression

HSPs were originally discovered in *Drosophila melanogaster*, in which puffs appeared on the giant

chromosomes of the salivary glands in response to heat exposure (Ritossa, 1962). Tissières et al. (1974) later identified novel protein synthesis in the salivary glands of *Drosophila* that was related to the chromosomal puffs induced by heat exposure. It was subsequently hypothesized that the denaturation of mature proteins inside the cell following stress exposure was the triggering event for increased protein synthesis (Hightower, 1980). As previously described, there are now abundant data to support this hypothesis.

Numerous studies have examined the effect of stressful stimuli on the time course of HSP gene expression in a variety of cell and tissue types. Schena et al. (1996) examined the heat shock response in human T cells (43°C for 4 h) and heart tissue using cDNA microarray technology and noted a significant increase in HSP 90 expression. In human PBMCs, maximal expression of intracellular HSP 70 was observed between 4 and 6 h after heat shock (43°C for 20 min; Sonna et al., 2002). Increased HSP gene expression (HSP 10, 20, 40, 60, 70, 90, and 110) has also been observed in response to exertional heat stroke and hypoxia in PBMC and human hepatocytes, respectively (Sonna et al., 2003, 2004, see Chapter 16 in this volume). *In vivo* approaches have also been successful in demonstrating increased HSP gene expression in response to heat and other stressors. Blake et al. (1990) showed that the ambient temperature to which rats were exposed, as well as the duration of exposure to a given temperature were factors that determined the magnitude of HSP 70 mRNA induction in brain, lung, and skin. Whether core body temperature was elevated by exposure to high temperature for a short duration or to a lower temperature for a longer duration, there was a direct correlation between maximum core temperature attained and the level of HSP gene expression (Blake et al., 1990). Flanagan et al. (1995) directly tested the effect of heating rate on the tissue-specific HSP 70 response in rats and showed that a high rate of passive heating (0.175°C/min) induced greater HSP 70 expression in the liver, small intestine, and kidney than a low rate of heating (0.05°C/min). The kinetics of HSP 70 induction has also been shown to differ by organ. In rats, brain, lung, and skin showed the most rapid induction (~1 h), whereas HSP 70 was maximally expressed in the liver at 6 h after heat exposure (Blake et al., 1990). Liver induction of HSP 70 has also been observed in rats 24 h following a 5–10 min exposure to ambient temperature of 40°C (Kluger et al., 1997). The time course of increased HSP 70 expression correlated with reduced TNF production and enhanced LPS-induced fever in heat stressed rats, supporting a role for HSP 70 in the modulation of core temperature responses (through an alteration of cytokine production) during infection. TNF has been implicated as an endogenous antipyretic, such that the inhibition of its production by HSPs would be expected to enhance fever responses (Kluger et al., 1997).

In the rat brain, the distribution of HSP 72 has been localized to neurons located in the dentate gyrus, medial habenula, hypothalamus, granular layer of the cerebellum, glia, endothelial cells of the arterioles, choroid plexus, and the olfactory area following *in vivo* hyperthermia that induced a core temperature elevation of 41.5°C (Li et al., 1992a). Increased HSP 72 expression in the hypothalamus and hippocampus suggests a direct connection between thermal injury and alteration of thermoregulatory control mechanisms, which are thought to reside in those brain areas. Focal cerebral ischemia had a similar effect on neuronal HSP 72 expression in the rat brain (Li et al., 1992b). In human fetal astrocytes, HSP 27 is constitutively expressed and shows increased phosphorylation following a 30 min exposure to heat shock, cytokines, or growth factors (Satoh and Kim, 1995). Importantly, it is now known that a "heat shock" paradigm is not needed to evoke the expression of HSPs in mammals. Heat acclimation for several weeks to a hot but not lethal environment is sufficient to elicit significant HSP expression. For example, rats maintained for 4 weeks at an ambient temperature of 34°C undergo a 175% increase in HSP 72 levels in cardiac tissue (Maloyan et al., 1999). The degree to which core or peripheral tissue temperatures must increase to elicit the HSP response is not clear. One might expect the threshold temperature for HSP induction to correlate with the temperatures in which a particular species normally lives (reviewed in

Feder and Hofmann, 1999). Although one would expect a significant elevation in core temperature of the rat when maintained continuously at 34°C, core temperature is commonly not measured in such studies (Maloyan et al., 1999).

Trauma is also sufficient to induce HSP 70 expression in rat brain (Brown et al., 1989; Gower et al., 1989). Thus, in addition to serving as a biomarker of thermal injury, HSP expression is useful for the identification of reactive cells that respond to trauma in the presumed absence of a core temperature change (Brown et al., 1989). Exercise is another potential stimulator of HSP induction, although the mechanisms that lead to the heat shock response are not fully understood. The etiology of exercise-induced effects on homeostasis has been attributed to increased core temperature, oxidative stress, accumulation of lactic acid, alteration in calcium homeostasis, and glucose deprivation (Salo et al., 1991; Kilgore et al., 1998; Clarkson and Sayers, 1999). Moderate intensity exercise induced HSP 70 expression in locomotor muscles of the rat hindlimb, despite maintenance of core temperature at baseline levels (Skidmore et al., 1995); however, muscle temperature was not measured so it is difficult to determine if a complete dissociation between exercise and heat effects was achieved in this study. Walters et al. (1998) measured exercise-induced brain mRNA and protein levels of HSP 70 in which brain temperature was maintained at baseline levels; a change in HSP expression was undetectable in seven fore- and midbrain regions or three hindbrain regions of the rat, suggesting that exercise alone was not sufficient for HSP induction in their model.

Thermotolerance and injury protection

Acquired thermotolerance is the term used to describe the non-inheritable, transient resistance to lethal heat stress that is acquired following an initial, short exposure to a non-lethal heat treatment. Several studies have demonstrated a temporal relationship between the development of thermotolerance and HSP expression, accumulation, and degradation (Landry et al., 1982; Li and Werb, 1982; Subjeck et al., 1982). Of the several types of HSPs that are synthesized in response to heat exposure, HSP 70 concentrations show the best correlation with thermotolerance. Li et al. (1991) used rat fibroblasts expressing different levels of a cloned HSP 70-encoding human gene to show that the higher the level of expressed HSP 70, the greater the thermal resistance of cells to a heat shock treatment of 45°C for 90 min. Na arsenite, hypoxia, and ethanol also induced tolerance to a subsequent heat exposure in Chinese hamster fibroblasts, which correlated with increased synthesis of HSP 70, 87, and 97 (Li and Werb, 1982). Thus, stressors in addition to heat can confer protection to subsequent thermal damage. Additional evidence in support of a role of HSP 70 in thermotolerance is provided by studies examining the effect of competitive inhibition or over-expression of HSP function. Johnston and Kucey (1988) showed that a 90% reduction of endogenous HSP 70 levels, achieved following the introduction of a dominant negative mutant HSP 70 gene into Chinese hamster ovary cells, increased thermosensitivity as illustrated by a more rapid time course of cell death and reduced colony formation compared to controls. In a similar manner, the introduction of affinity-purified monoclonal antibodies to HSP 70 into rat fibroblasts rendered those cells incapable of surviving a 30 min heat shock at 45°C, a response that correlated with a loss of cell membrane integrity (Riabowol et al., 1988). As an important control, heat-denatured HSP 70 antibodies as well as control antibody injections had no effect on cell survival, indicating that antigen:antibody complexes were not responsible for the observed change in thermosensitivity. Further analysis revealed that increasing dilutions of injected HSP 70 antibodies resulted in an increase in thermal cytotoxicity, indicating that the level of thermotolerance was directly dependent on the concentration of HSP 70. Finally, cells microinjected with the HSP 70 antibody showed low or absent levels of nuclear staining for the protein, suggesting that nuclear translocation (and presumed effects on gene transcription) was necessary for the thermotolerant effects observed by prior heat treatment in control cells.

Due to a reduction of ATP levels during ischemia, protein translation is halted; the protective

effect of HSP 70 under these conditions is thought to reside in their ability to assist in the refolding of denatured proteins following commencement of reperfusion. An in situ study of the hearts of transgenic mice overexpressing rat HSP 70 protein showed a 40% reduction in the zone of infarction, which coincided with a two-fold increase in contractile function following ischemic injury (Marber et al., 1995). Unfortunately, this study was conducted in a non-functional, buffer per-fused heart, making applicability to the *in vivo* condition questionable. However, Hutter et al. (1996) confirmed this result in transgenic mice overexpressing HSP 72 in brain, cardiac, and skel-etal muscle by showing significantly decreased inf-arct size following a 30 min left coronary artery occlusion. Similar results have been reported in response to cerebral infarction (Rajdev et al., 2000). Cross tolerance, in which induction of HSPs from one stress agent confers protection against a different subsequent insult, provided myocardial protection against ischemia (Hutter et al., 1994; Stojadinovic et al., 1995), phospholipase A_2- and sepsis-induced acute lung injury (Villar et al., 1993, 1994), and heat stroke and endotoxin mortality (Ryan et al., 1992, 1994; Hotchkiss et al., 1993; Lappas et al., 1994; Yang et al., 1998). Thus, the term "thermotolerance" is a bit of a misnomer since tolerance can be acquired by pretreatment with agents other than heat, such as Na arsenite, as long as those treatments are able to induce HSP synthesis. Furthermore, increased HSP 70 expres-sion can protect against a variety of stressors in additional to thermal toxicity.

Heat shock proteins and cytokines

HSP gene expression is mediated primarily at the level of gene transcription by a family of heat shock transcription factors (HSF) that interact with a regulatory element, known as the heat shock element (HSE), in the promoter region of genes. The major stress responsive HSF in mam-malian cells is HSF-1, which is constitutively present in a non-DNA binding state, but is rap-idly transformed to a nuclear form following exposure to heat or other stresses. Subsequent to nuclear translocation, HSF-1 binds to heat shock elements (HSEs) to regulate gene transcription (Voellmy, 1994; Wu, 1995). Under stressful con-ditions, gene promoter activity is significantly in-hibited. A hypothesis to account for this response is that the inhibition of transcription serves to limit the accumulation of nascent or denatured proteins until more favorable environmental conditions are restored.

Mechanism of HSP protection: inhibition of cytokine transcription

The inhibition of IL-1, IL-6, and TNFα produc-tion provides protection against the morbidity and mortality of endotoxin exposure in several experimental models (Silva et al., 1990; Lundblad et al., 1995; Luheshi et al., 1996; Leon et al., 1998). HSF-1 is activated by febrile-range temperatures and the interaction of HSF-1 with HSEs in the promoter region of cytokine genes is a potential mechanism by which HSPs can inhibit cytokine gene transcription and confer protection against endotoxin and other infectious/inflammatory stim-uli. Several studies have shown a direct effect of stress-induced HSP production on cytokine levels. In gene-transfected human PBMCs, overexpres-sion of HSP 70 significantly reduced LPS-induced (*Brucella melitensis*) production of TNFα, IL-1β, IL-10, and IL-12, an effect that was reversible following treatment with antisense HSP 70 treat-ment (Ding et al., 2001). Others have shown opposite effects on pro- and anti-inflammatory cytokines, with a decrease in LPS-induced IL-12 (pro-inflammatory) levels and increase in IL-10 (anti-inflammatory) levels following heat exposure (Wang et al., 2001b). Differences in cytokine pro-files may have been related to the time of cytokine measurement following heat shock treatment (4 h vs. 24 h), which complicates inter-study comparisons in most cases.

Interestingly, HSP 70 expression has been shown consistently to be without effect on IL-6 levels (Ensor et al., 1994, 1995; Ding et al., 2001; Wang et al., 2001b). The maintenance of IL-6 lev-els may be an indirect mechanism of HSP-induced inhibition of IL-1 and TNF, since IL-6 is a

negative regulator of these cytokines (a proposed anti-inflammatory effect of IL-6; Ding et al., 2001). The downregulation of IL-1 and TNF production has been demonstrated in a variety of heat shock models. Heat shock treatment, administered at several different time points prior to LPS stimulation was effective in down-regulating TNFα and IL-1β production in human and rat macrophage, Kupffer and glomerular cells (Velasco et al., 1991; Fouqueray et al., 1992; Snyder et al., 1992; Ensor et al., 1994). Not surprisingly, the protective effect of heat treatment was dependent on the dose of LPS — the higher the LPS dose, the less effective the heat treatment or the higher temperature required to confer protection (Ensor et al., 1994). Importantly, a time course of heat-induced HSP expression was temporally correlated with cytokine inhibition in most studies — thus, it is not heat *per se*, but stressor-induced HSP production (i.e., heat, Na arsenite, etc.) that is mediating cytokine inhibition. In a human monocytic cell line, heat shock (42.5°C for 30 min) induced HSP 70 mRNA and a consequent inhibition of LPS-induced IL-1β and TNFα transcription (Xie et al., 2002). This heat shock effect was mediated through direct interaction of HSF-1 with the nuclear factor of IL-6 (NF-IL6, also known as a CCAAT enhancer binding protein or C/EBPβ), which is a direct regulator of IL-1β transcription (Xie et al., 2002). That is, HSF-1 bound to NF-IL6 to inhibit its ability to interact with the IL-1β promoter (Xie et al., 2002). Transcriptional control of TNFα has also been demonstrated — overexpression of HSF-1 or exposure to febrile range temperatures reduced TNFα promoter activity in macrophages (Singh et al., 2000). The relevance of transcriptional regulation of cytokine repression to the *in vivo* condition is demonstrated in HSF-1 knockout mice, which show increased mortality and an exaggerated TNFα response after endotoxic challenge (Xiao et al., 1999).

Aging and heat stroke

At the time of the writing of this chapter, Hurricane Katrina ravaged the Gulf Coast of the United States and, due to the destructive forces of the storm on electrical power supplies, caused significant mortalities of the elderly as air conditioning units were rendered inoperable in the face of rising local temperatures (as high as 110°F were reported; *NY Times*, Sept. 19, 2005). Even if the implementation of cooling or hydration strategies could have been implemented in the aftermath of this destructive storm, the presence of pre-existing illnesses in the elderly population of this region (many of the elderly resided in nursing homes) may have rendered them unresponsive to medical treatment. Clearly, a more thorough understanding of age-associated alterations in heat-induced responses is required for proper diagnosis, prevention, and treatment of this unique population.

Hyperthermia, fever, HSP expression, and cytokine production are all hallmarks of heat exposure that may be altered in aged individuals, an effect that may account for increased heat stroke morbidity and mortality in this population. It is expected that during the life of an organism there is an accumulation of protein damage resulting from continual oxidant/free radical activity. As stress tolerance deteriorates with aging, due to a variety of compromised mechanisms, an organism becomes less able to mitigate the adverse effects of protein denaturation with a resultant increase in stress morbidity and mortality (Holbrook and Udelsman, 1994; Lee et al., 1996). Alzheimer's disease has been postulated to be a consequence of decreased HSP function, which results in increased deposition of abnormally folded proteins (Morrison-Bogorad et al., 1995). In *Drosophila*, the original organism in which HSP were discovered, lifespan is extended by heat shock treatment or following the addition of HSP 70 gene copies, suggesting that an increase in protein chaperonin activity provides protection during aging (Khazaeli et al., 1997; Tatar et al., 1997). As suggested by Kim (2003), the identification of individuals with high HSP antibody titers might be useful in the identification of those particularly susceptible to heat stroke (or other stressors).

Several studies have reported altered heat stress responses with aging. In rats, aging was associated with a significant reduction in liver HSP expression following passive heat exposure (Kregel and Moseley, 1996). However, the ability of aged rats

to show a similar HSP 70 response as mature rats to an exertional heat load suggested that the response to passive heating was not due to a global inability to express HSP; rather, a reduction in the threshold for HSP stimulation appeared to have changed during the aging process (Kregel and Moseley, 1996). Similarly, Fargnoli et al. (1990) showed that a global reduction in protein synthesis was not responsible for decreased HSP induction in aged lung fibroblasts. Overall protein synthesis patterns were similar between old and young cultures before and after heat stress (including HSP 27), but HSP 70 was significantly reduced with aging. One of the confounding variables in many studies is the lower core temperature increase induced in aged vs. mature rats during heat exposure. For example, aged rats showed a significant reduction in HSP 70 mRNA levels with heating, but it is unclear in many studies if this is a consequence of a lower heating rate, lower starting core temperature, and/or lower final core temperature in aged compared to young rats (Blake et al., 1991).

Several studies have reported impairment of baroreceptor reflex modulation (Stauss et al., 1997), lower sweating rate and longer onset to sweating (Inoue et al., 1999), and diminished renal and splanchnic sympathetic nerve discharge in aged organisms (Kenney and Fels, 2002). Minson et al. (1998) demonstrated that older men relied on a higher percentage of their cardiac chronotropic reserve compared to young men during heat exposure. This finding may have particular relevance to those individuals experiencing a heat wave with a pre-existing condition, such as coronary artery disease (Minson et al., 1998). One would expect a greater risk for a cardiac event under those conditions, as a higher heart rate would be required to maintain cardiac function in a hot environment. Additional factors to consider with aging is that the time course of HSP expression may differ in young and old rats, such that analysis at only time point may not be adequate to determine alterations in expression profiles in this population (Kregel and Moseley, 1996). Differences in body size are also important to consider. Walters et al. (2001) maintained constant body mass differences and heating profiles of aged and young rats to

assess effect of heat on the time course of HSP 70 expression in the CNS — they demonstrated a time-dependent and regionally specific alteration of HSP 70 expression in aged rats.

An elevation in core temperature, whether due to an external heat load or internal generation of fever, has been shown to induce HSP synthesis *in vivo*. As described previously in this chapter, hyperthermia, hypothermia, and fever are common thermoregulatory responses to heat exposure that may be regulated by endogenous HSP and/or cytokine interactions in the host. Several studies have shown decreased fever responses in aged rats in response to IL-1β, IL-6, endotoxin, and prostaglandin (the final mediator of fever) injections (Norman et al., 1988; Miller et al., 1995; Satinoff et al., 1999; Krabbe et al., 2001; Buchanan et al., 2003; Peloso et al., 2003). In some cases, cytokine profiles in response to endotoxin were altered in the aged. For example, aging was associated with a prolonged fever and higher TNFα:IL-10 ratio following endotoxin injection in humans (Krabbe et al., 2001). Thus, an exaggerated pro-inflammatory cytokine response was sustained in these individuals, suggestive of an initial hyperreactivity and delayed termination of the APR in this population (Krabbe et al., 2001). Altered mechanisms of fever development in the aged may range from reduced sensitivity of endogenous pyrogen receptors, altered cytokine expression, changes in BBB permeability that reduces cytokine entrance into the brain to initiate an increase in the thermal setpoint, or changes in heat production capabilities (Chorinchath et al., 1996; Tateda et al., 1996; McLay et al., 2000; Buchanan et al., 2003; Peloso et al., 2003). Interestingly, access to warm ambient temperatures facilitates the development of endotoxin-induced fever in aged rats (Peloso et al., 2003). Thus, as shown with mice during heat stroke recovery, the ambient temperature that an organism experiences during manifestation of SIRS can have a profound impact on thermoregulatory responses and recovery. These effects in the aged may have profound consequences on heat stroke mortality rates in that the absence of hyperthermia or fever in the aged may prevent proper heat stroke diagnosis, and altered cytokine and thermoregulatory (hypothermia/fever) responses may exacerbate and/or prolong heat-induced SIRS.

Conclusion

As the average lifespan of the human population and the incidence of global warming increases, a rise in the incidence of heat stroke mortality may be anticipated. Current health care strategies for the prevention and/or treatment of heat stroke focus on the implementation of hydration guidelines, acclimatization protocols, and rapid cooling therapies. However, despite successful implementation of these techniques, significant adverse consequences of prolonged heat exposure continue to be realized. The fact that many heat stroke survivors incur permanent neurological damage despite cooling therapy suggests that current knowledge of the mechanisms responsible for the adverse consequences of this syndrome are not fully understood. Perhaps the most important outcome to-date of the study of heat stroke pathophysiology has been the realization that it is a "syndrome" that encompasses not only the responses elicited during direct heat exposure, but also those that ensue during long-term recovery. Given the current implication of cytokines in the pathophysiology of heat stroke and improvements in experimental techniques (e.g., radiotelemetry, transgenic technologies, stem cell therapy, etc.), it is anticipated that rapid advancements will be made in the near future in our understanding of the role of cytokines, and other physiological mediators, in the morbidity and mortality of this syndrome.

Abbreviations

5-HT	serotonin
5,7-DHT	5,7-dihydroxytryptamine
6-OHDA	6-hydroxydopamine
ADX	adrenalectomy
ALT	alanine aminotransferase
APR	acute phase response
AST	aspartate amino transferase
BBB	blood brain barrier
CLP	cecal ligation and puncture
CNS	central nervous system
CPK	creatine phosphokinase
CRP	C-reactive protein
CTM	critical thermal maximum
DA	dopamine
DIC	disseminated intravascular coagulation
GI	gastrointestinal
HPA	hypothalamic-pituitary-adrenal axis
HSP	heat shock protein
ICP	intracranial pressure
IFI	interferon inducible
IFN	interferon
IL	interleukin
IL-1ra	interleukin-1 receptor antagonist
IL-2R	interleukin-2 receptor
IPH	intraperitoneal heating
LPS	lipopolysaccharide
MAP	mean arterial pressure
MCP-1	monocyte chemoattractant protein-1
PAI-1	plasminogen activator inhibitor
PBMC	peripheral blood mononuclear cells
sIL-6R	soluble interleukin-6 receptor
siRNA	small interfering RNA
SIRS	systemic inflammatory response syndrome
sTNFR	soluble tumor necrosis factor receptor
T_a	ambient temperature
T_c	core temperature
$T_{c,Max}$	maximum core temperature
T_{es}	esophageal temperature
TF	tissue factor
T_h	hypothalamic temperature
TNF	tumor necrosis factor
tPA	tissue plasminogen activator
T_{re}	rectal temperature
WBH	whole body hyperthermia

official or reflecting the views of the Army or the Department of Defense. In conducting the research described in this report, the investigators adhered to the "Guide for Care and Use of Laboratory Animals" as prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. Any citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement of approval of the products or services of these organizations.

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